

# Preferred visually evoked spatial and temporal frequencies in primary visual cortex of alert and anesthetized rats

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Sandra Nøkkevangen



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## **ABSTRACT**

The primary visual cortex is the first cortical area in the visual processing pathway. Neurons in the primary visual cortex respond well to sinusoidal drifting gratings across a range of different spatial and temporal frequencies. Most studies of single neurons in rat primary visual cortex have been conducted during anesthesia. However, anesthesia has extensive impact on the brain and it is therefore debated whether visual properties registered in anesthesia are applicable for the alert state.

The aim of this study was therefore to investigate preferences in spatial and temporal frequencies for single units in primary visual cortex in alert rats and compare these findings with preferences found for the same units during anesthesia. This was done by measuring single unit activity using chronically implanted electrodes while the alert rat was presented stimuli of different spatial or temporal frequencies. The same experiments were repeated during anesthesia.

The results show a decrease in spiking activity of single units of approximately 50% during anesthesia. Preferred spatial and temporal frequencies in anesthetized state were in line with previous findings. In alert state, on the other hand, single units showed a tendency towards stronger response to stimuli of higher spatial frequencies. When comparing stimuli of different temporal frequencies, we did not detect any difference between the preferred stimuli in the alert and anesthetized state.

The preference of higher spatial frequencies in the alert state indicates that the alert animal is able to discriminate finer details and illustrates that experiments from alert animals is important in order to understand visual processing.

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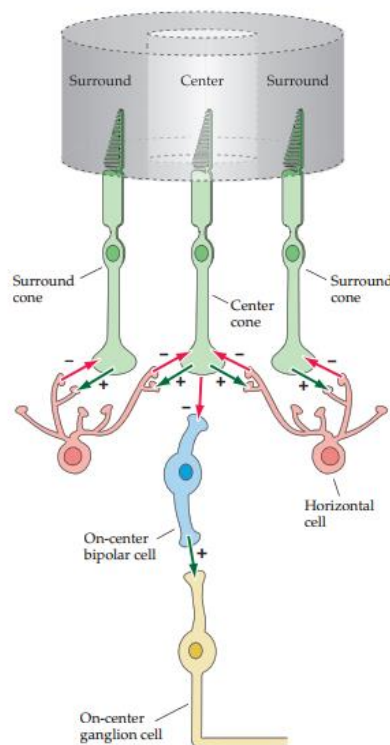
# **1 INTRODUCTION**

Variations in light reflected from all parts of our physical world create an image that is represented and processed in our visual system. In mammals the visual cortex is essential for processing of visual input from the eyes. The region is divided into different areas and this study will focus on the primary visual cortex (V1), which is considered the first cortical area of the visual processing pathway. To gain information about the operating principles of the human brain, and diseases mechanisms, rats are often used as model organisms. There are lots of different genetic tools and strains available for rodents. In addition they have a relatively short generation time and are easy to breed. Rats are used in particular since they are easily trained. Furthermore the rat visual system, although smaller and less organized, share many features with human visual system which enable comparisons across species. It is therefore important to elucidate the properties of rat vision. The functional properties of visual cortical neurons are essential knowledge for further study of the rat as a model. Little is known about the properties of single neurons in rat primary visual cortex, and most of the studies that are done have been conducted during anesthesia (Girman et al. 1999). Anesthesia has an extensive impact on cortex and consciousness in humans (Greenberg et al. 2008, Brown et al. 2010, Alitto et al. 2011), and therefore it is debated whether visual properties registered in anesthesia are applicable for the alert state (Wurtz 1969).

## **1.1 The Visual pathway**

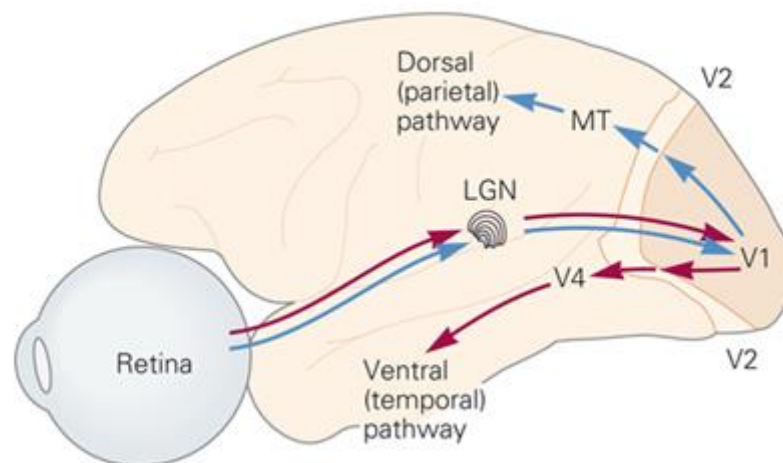
Receptor cells on the retina have graded potentials and synapse directly onto bipolar cells as well as horizontal cells. Bipolar cells are connected to ganglion cells which are the first to produce action potentials and their axons project into the brain. Horizontal cells synapse only onto bipolar cells. In this way there is an indirect pathway from receptors to bipolar cells, in addition to the direct one. The direct pathway from receptor to bipolar cell can be either excitatory or inhibitory. The indirect pathway is always opposite of the direct one (Figure 1).

Ganglion cells have circular receptive fields and there are two distinct types of cells; On-center cells respond to light at the center of the receptive field. Off-center cells respond inhibitory to light at the center of the receptive field. In both cases, the area surrounding the receptive field center does always have the opposite property (Palmer 1999).



**Figure 1: Early visual pathway and receptive fields.** Ganglion On-center cells will respond excitatory to light at the center of the field and inhibitory to light at the surround. The opposite is true for off-center cells (Purves et al. 2008).

Ganglion axons exit the eye through the optic nerve, and protrude to the Lateral Geniculate Nuclei (LGN) of thalamus. On the way, they pass through the optic chiasm, where some of the axons cross to the opposite side. The cells in the LGN have receptive fields that are similar to those of retinal ganglion cells, but they are larger and the inhibitory surround is stronger. The LGN consists of two-dimensional sheets of neurons, and are laminar. It has six layers of cells which are folded. The lower two layers consist of magnocellular neurons, the upper four layers consists of parvocellular neurons (Palmer 1999). The magnocellular and parvocellular neurons have different properties and the separation of information in different pathways with parallel processing, is a hallmark of the visual system.



**Figure 2: Visual pathway in humans.** Visually guided movement is mainly taken care of by the dorsal pathway. This is where cells selective for direction of movement are found. The ventral pathway is associated with object recognition (Kandel et al. 2000).

Since the current study is going to focus on how visual cortical neurons respond to stimuli of different spatial and temporal frequencies, it is important to be aware of how neurons in LGN contribute to such specialization. The magnocellular neurons are relatively large with large receptive fields, and exhibit low color sensitivity, high contrast sensitivity, low spatial resolution and fast temporal resolution. Parvocellular neurons, on the other hand, are quite small with small receptive fields. They are sensitive to color, but not so much to contrast. Their spatial resolution is high and temporal resolution is slow. It is important to be aware that these distinctions are not clear-cut. There is substantial overlap between magnocellular and parvocellular neurons. Some scientists have speculated that magnocellular neurons are mainly responsible for processing of motion and depth, whereas parvocellular neurons are mainly responsible for processing color and shape (DeYoe and Van Essen 1988, Livingstone and Hubel 1988).



**Table 1:** Properties of parvocellular and magnocellular cells.

	<b>PARVO</b>	<b>MAGNO</b>
Color sensitivity	Higher	Lower
Contrast sensitivity	Lower	Higher
<b>Spatial frequency</b>	<b>Higher</b>	<b>Lower</b>
<b>Temporal frequency</b>	<b>Lower</b>	<b>Higher</b>
Receptive field size	Small	Large

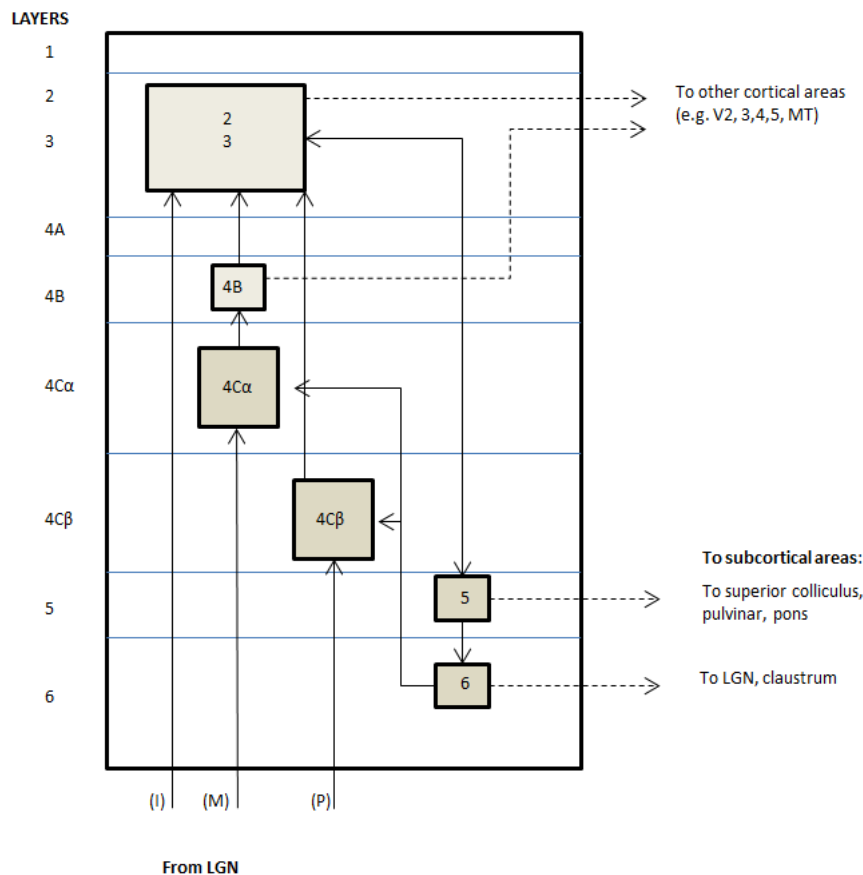
As a whole, the LGN receives input from both eyes, but each layer receives information from only one eye. LGN axons protrude to the primary visual cortex, layer 4, through the optic radiations.

The visual pathway and receptive field properties of cells in visual cortex have been extensively studied in non-human primates and carnivores such as cat. However, it is mainly the same for rats except rats do not have any fovea, though it has been observed conjugate saccadic eye movement when stimulating superior colliculus (SC). Rats have also have laterally placed eyes with a limited binocular overlap (Sefton et al. 2004). Cells in rat LGN with similar properties as M-cells and P-cells are often referred to as X-like and Y-like cells.

## **1.2 Visual cortex**

Primary visual cortex (V1) is the initial site of cortical processing of visual sensory information. It is divided into six cell layers: 1, 2/3, 4B, 4C $\alpha$ , 4C $\beta$ , 5 and 6, listed in dorsal-ventral direction. It consists mainly of pyramidal cells and non-pyramidal cells which could be spiny stellate or smooth stellate. When information from LGN reaches layer 4 in the V1, it flows from one cortical layer to another. The input from LGN is distributed to the cortex by projecting spiny stellate cells. Further pyramidal cells extend axon collaterals upwards and downwards for integration of the activity within the layers. Pyramidal cells also projects to cortical areas outside of V1 (V2, V3, 4, 5, Medial Temporal cortex) and areas outside visual cortex (superior colliculus, pulvinar, pons, LGN and claustrum) (Figure 3) (Kandel et al. 2000).

The projections from the primary visual cortex to other areas of the brain run along two main routes of information processing. These are referred to as the Dorsal stream – running from the V1 to the parietal cortex, and the Ventral stream – running from the V1 to the temporal cortex (Figure 2). These have been found to process very separate aspects of visual information. The dorsal pathway is primarily involved in visual movement (spatial vision), with responses similar to M-cell properties, and is more associated with temporal frequencies. On the other hand the ventral stream has been found to process information more similar to P-cell responses, with higher selectivity for patterns and objects (object recognition) and is more associated with spatial frequencies. These have been referred to as the “where” and the “what” pathways because it is believed that they process two very different aspects of the visual world, the location and movement of objects versus the identity and memory of objects. How these pathways are processed and divided in the V1 is still largely unknown (Purves et al. 2008), but it has been found that the magnocellular and parvocellular pathways terminate in different sub layers of V1 layer 4 (Kandel et al. 2000).



**Figure 3: Flow of information across layers in primary visual cortex (V1).** Axons from LGN have three main sources and destinations. Intralaminar regions in LGN (I) project to layer 2/3(I), whereas M-cell (M) and P-cells (P) project to sub layer 4C. Layer 4C projects axon collaterals to layer 4B or 2/3. Layer 2/3 projects to layer 5 which in turn projects to both layer 2/3 and 6. Layer 6 is sending axon collateral to layer 4C. Output projections to other cortical areas come from layer 2/3 and layer 4B. Output projections to subcortical areas come from layer 5 and 6. (Figure is based on figure from(Kandel et al. 2000).

### 1.3 Electrophysiology of the primary visual cortex

Much is known about the electrophysiology of neurons in the visual cortex. When Hubel and Wiesel (Hubel and Wiesel 1959) applied the novel receptive field mapping technique (Kuffler 1953) to the primary visual cortex (V1) they made a surprising discovery. To activate the cells they were recording from, they had a spot on a microscope slide which they moved in front of a projector. Sometimes the cell burst with action potentials. They discovered that it was not the spot that triggered the response, but the shadow of the slide's edge that moved in a particular direction across the retina in a particular position. From these experiments they concluded that neurons in the visual cortex respond preferentially to bars of light at a particular orientation. Colleagues elaborated on their findings and established that neurons

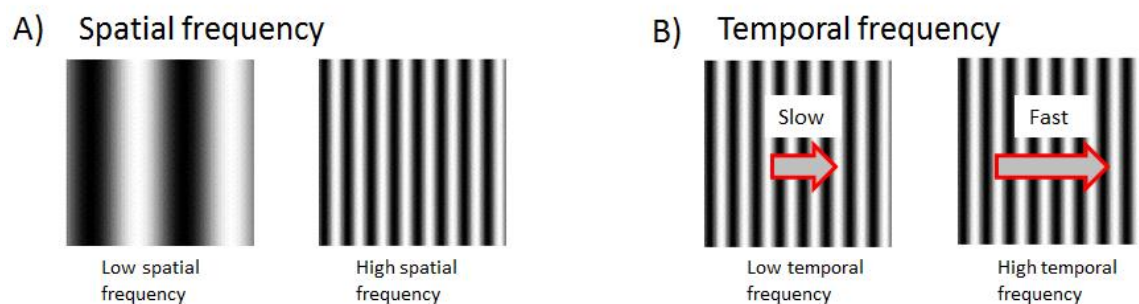
in the visual cortex respond optimally to sine wave gratings of different spatial frequencies rather than simple bars and edges (Albrecht et al. 1980)

From these experiments Hubel and Wiesel (1959) described three different cortical cells with different receptive field characteristics; simple cells, complex cells and hypercomplex cells. Simple cells respond to spots of light, and the response to complex stimuli can be predicted by summing the responses from the spots of light that compose it. It is believed that because of these findings, an early step of visual processing is finding lines and edges. Simple cells can have many different receptive field sizes. However, about 75% of the visual cells in V1 are complex cells. They differ from simple cells in many ways; Complex cells are not linear, and they have little or no response to small stationary spots. Moving lines or edges anywhere within the receptive field increase the firing rate from complex cells. The motion sensitivity is often specific to one (or two) directions. The position of a certain stimuli in the receptive field is not very important for complex cells and does not affect the firing rate. Complex cells have, on average, larger receptive fields than simple cells. Some complex cells receive input from the LGN, but it is believed that they mostly receive input from many simple cells. Hypercomplex cells have highly selective receptive fields. Some hypercomplex cells are end-stopped cells. These cells fire less when a line or edge is extended beyond a certain length. It is proposed that hypercomplex cells actually are end-stopped simple or complex cells, instead of being one distinct cell type (Kandel et al. 2000). Half of the population of cells will respond to a flashing, uniform and stationary on-off stimulus. Complex or end-stopped receptive fields are only found in pyramidal cells. Pyramidal cells with complex receptive fields are found in layers 2 to 4 and pyramidal cells with end-stopped cells are found in layers 2/3 and 5. Cells with simple receptive field are found in layers 2/3 and 4 (Sefton et al. 2004).

#### **1.4 Spatial- and temporal frequency**

Neurons in the visual cortex respond more strongly to sinusoidal gratings of different spatial frequencies rather than simple bars and edges (Albrecht et al. 1980). Sinusoidal gratings are patterns where the luminance vary according to a sine wave in one direction, but are constant over the perpendicular direction.

Each sinusoidal grating can be described from four parameters; its spatial frequency, orientation, amplitude and phase. Spatial frequency is measured in cycles/degree of visual angle, and it is a description of the number of repetitions of a pattern over a given distance. In other words will low-frequency gratings have thick bars, and high-frequency grating have thin bars. Since spatial frequency measures the number of repetitions per degree of visual angle, it is important to keep in mind that the distance between stimuli and viewer will also contribute to the actual spatial frequency. Since it has been shown that sinusoidal drifting gratings elicit the highest response of neurons in V1 (Albrecht et al. 1980), this is the stimuli used in this project.



**Figure 4: Sinusoidal drifting gratings.** A) Low versus high spatial frequency. B) Low versus high temporal frequency.



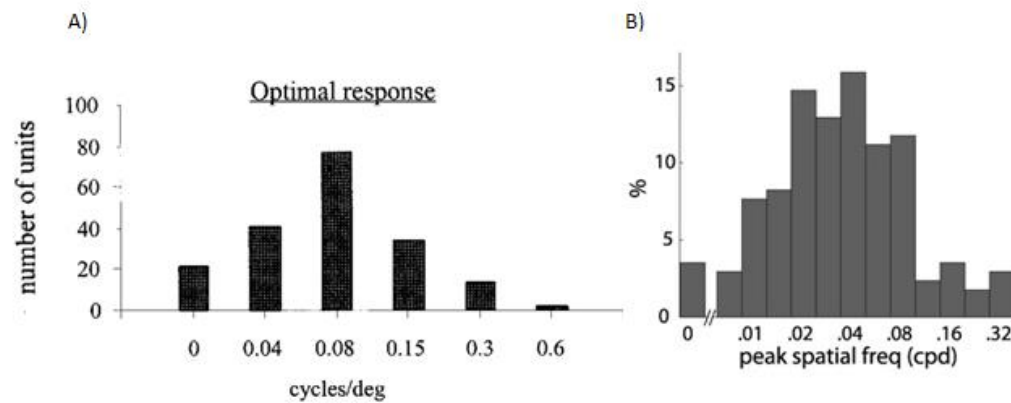
**Figure 5:** Visual stimulus as changes in light intensity. (Figure from Albrecht et al. 1980)

Temporal frequency of visual gratings is measured as cycles per second and describes how rapidly the pattern changes over time. Thus, a grating pattern where four cycles of black and white passes a particular point per second have a temporal frequency of 4 Hz. The temporal frequency will be higher if the zebra is running past the observer than if it is walking.

### **1.5 Preferred spatial and temporal frequency in rodents**

Neurons in V1 in rodents show similar visual response properties as other mammals, but they have an overall increase in receptive field sizes (Girman et al. 1999, Niell and Stryker 2008). Girman and colleagues (1999) report that neurons in the visual cortex of the rat, respond stronger to some spatial- and temporal frequencies in anesthesia. They also found that neurons are tuned for orientation and direction, that there is orientation tuning of surround modulation and contrast sensitivity. However my study and the following description will be concentrated on the findings in spatial and temporal frequencies. Girman et al. (1999) found that rat V1 neurons are sensitive to spatial frequencies of  $\leq 1,2$  c/degree. On average neurons were tuned to relatively low spatial frequencies and the majority had sharp spatial frequency tuning. They also found that neurons in rat V1 cortex responded best to high temporal frequencies. The preferred temporal- and spatial frequency did also vary across layers in V1. These results show that the response properties of V1 neurons in rats are well tuned, and they are as specialized as those of highly visual animals (Girman et al. 1999).

From brain lesion studies it is concluded that V1 in rats is responsible for discrimination of spatial frequencies up to approximately 1 c/d (Dean 1981).



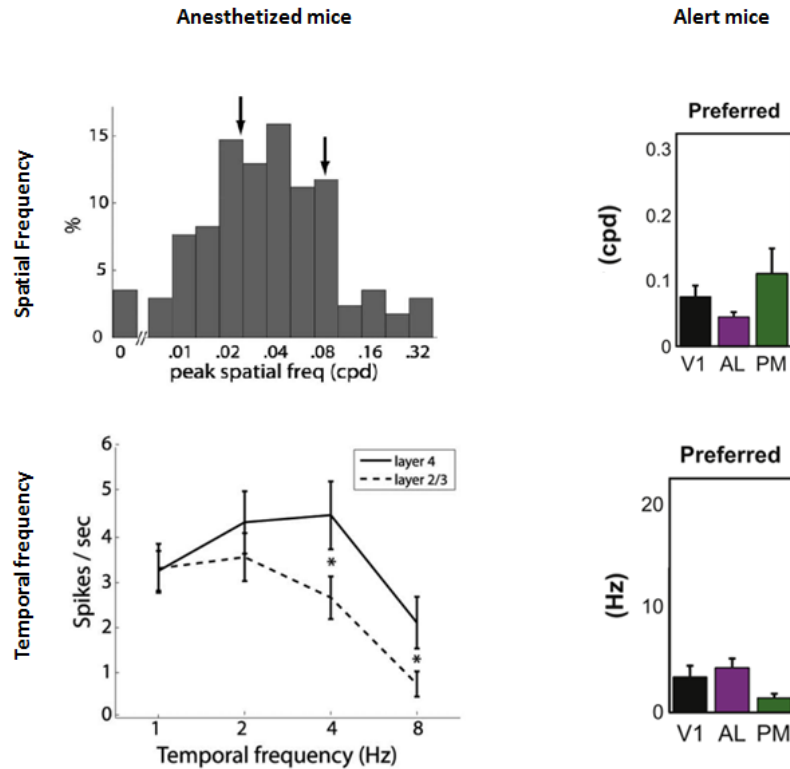
**Figure 6: Previously reported spatial frequency preference.** A) Optimal response of 0,08c/d in rats (Girman et al. 1999). B) Peak spatial frequency of 0,04c/d in mice (Niell and Stryker 2008).

Spatial- and temporal frequency preferences have also been investigated in anesthetized mice (Niell and Stryker 2008). Neurons in V1 responded preferentially to gratings only over a particular range of spatial frequencies from 0,02c/d to 0,08c/d, and the highest frequency they tested was 0,32 c/d, which several units responded optimally to. In cats it is found a systemic variation in preferred spatial frequency across layers (Maffei and Fiorentini 1977), this is not found in mouse V1, except for layer 6 which responded optimally to lower spatial frequencies. Putative inhibitory units did also respond optimally to lower spatial frequencies. Neurons responded strongest to temporal frequencies between 2 and 4 Hz (Figure 7, right panels) (Niell and Stryker 2008).

The difference between the spatial frequency reported as maximum in anesthesia in the two species is thus 0,08c/d and 0,04c/d, suggesting that rats respond to slightly higher spatial frequencies than mice (Figure 6).

Two-photon calcium imaging in alert mice investigating visual responses in V1 and the downstream target areas AL (anterolateral) and PM (posteromedial) found that simultaneously imaged cells in V1 had different stimulus preferences. Evoked responses from many neurons were followed for several hours and this was used to estimate the

spatial and temporal frequency tuning for individual neurons. From this estimate, the spatial frequency preference was slightly less than  $>0,1\text{c/degree}$ , and temporal frequency preference was  $>4\text{ Hz}$  in layer 2/3 (Figure 7 left panels) (Andermann et al. 2011).



**Figure 7: Difference in response to spatial and temporal frequencies in alert and anesthetized mice.** Figures shown to the left are responses from anesthetized mice (Niell and Stryker 2008). Figures shown to the right are responses registered in alert mice. Purple and green columns indicate responses from other areas of the brain, and are not relevant in this comparison (Andermann et al. 2011)

Alitto et al. (2010) have reported response properties of neurons in the LGN of the thalamus in alert macaque monkey. They have also compared these responses to anesthetized animals. They found that both magnocellular and parvocellular neurons had a greater preferred temporal frequency in the alert state than in anesthetized state. Preferred spatial frequency for magnocellular neurons was also higher in alert animals than in anesthetized animals. In other words, alert animals can follow stimuli drifting with higher temporal and spatial frequencies than anesthetized animals. This is accompanied with an overall increase in firing rate in alert animals (Alitto et al. 2011).



## 1.6 Anesthesia and effects of anesthesia

In studies of the visual system, it has been most convenient to use anesthetized animals. To minimize confounding factors impacting the results the animal has to be completely still, especially when testing for orientation and direction tuning. It is now widely recognized that properties of cortical processes might differ between alert state and anesthesia (Andermann et al. 2011). On a general basis anesthesia alters the electroencephalogram (EEG) from high-frequency, low-amplitude to low-frequency, high-amplitude activity (Brown et al. 2010).

Metabolism is reduced in all areas as measured with PET, indicating reduced activity.

General anesthesia is also shown to reduce functional connectivity (Peltier et al. 2005). This is an indication that studies done in the anesthetized state are not necessarily applicable to the alert state. There have been few studies on visual responses in V1 of alert rats.

There has been extensive focus on studying how the most used anesthetic agents actually work in the brain (for review, see (Franks 2008)). Considering that a variety of different pharmaceuticals that act on different receptors all can achieve anesthesia, it is not likely that one particular receptor is responsible for anesthesia induction. However, some receptors have been found to be more involved than others. On a general basis, anesthetic agents alter the neurotransmission in cerebral cortex, brain-stem and thalamus (Brown et al. 2010), mainly by interaction with one or more of the following: GABA<sub>A</sub>-receptors ( $\gamma$ -aminobutyric acid, type A), 2PK-channels (two-pore-domain K<sup>+</sup> channels) or NMDA-receptors (N-methyl-D-aspartate) (Franks 2008). GABA<sub>A</sub>-receptors are found throughout the central nervous system (CNS) and induce Cl<sup>-</sup>-currents into the cell. Some anesthetics act as agonists at this receptor and activate GABA<sub>A</sub>-receptors in the absence of GABA and in this way inhibit action potentials or they could potentiate the effect of GABA to increase its effect. Activation of the 2PK channels has also been found with some anesthetics, which hyperpolarize the membranes or increase the membrane conductance. This will inhibit neuronal activity. In addition, a few anesthetics are antagonist to the NMDA-receptors, which are connected to excitatory neurotransmitters (Franks 2008). The anesthetics used in this project, Isoflurane and Midazolam, are both primarily GABA<sub>A</sub> agonists.

There is no imaging technique that can measure neuronal activity directly. The techniques used, utilizes changes in blood flow, glucose metabolism or oxygen concentration to infer changes in activity. PET is one such technique which has shown a reduction in cerebral blood

flow when consciousness is lost, however there is not a uniform deactivation; some regions are more deactivated than others, such as the thalamus. Thalamus is considered the main gateway between sensory information and cortex (Franks 2008).

From thalamus there are many projections to the cortex, referred to as “thalamocortical projections”. Individual thalamic nuclei modulate the activity of specific cortical regions via these thalamocortical projections. With regards to the visual pathway, the lateral geniculate nuclei (LGN) of the thalamus modulate the activity of the visual cortex. The cortex also project back to thalamus, referred to as “corticothalamic projections”, and these outnumber the thalamocortical projections. In the alert state, the corticothalamic projections depolarize the thalamocortical neurons. According to one theory this could prevent them from going into a synchronized, oscillatory state. Anesthetics might act as inhibitors of the cortical neurons that innervate the thalamic neurons. This will give rise to burst firing of the thalamocortical neurons, and correlates with a sleep like state in the animal. It has been reported that during awake states the thalamocortical projections has a tonic firing mode onto their synaptic targets, while in anesthesia this firing mode changes to a more phasic and synchronized pattern (Franks 2008). Whether this phasic pattern is governed by the anesthetic depression of corticothalamic projections is a topic for debate, but there is agreement that a tonic firing mode from thalamocortical neurons favors transmission of somatosensory information and a bursting firing mode inhibit the transmission of this information (Franks 2008, Brown et al. 2010). Anesthetics could also act as inhibitor of the excitatory arousal pathways or enhance the sleep pathways that control the thalamocortical or corticothalamic neurons (Franks 2008).

Anesthesia may influence many aspects of the visual process, and little is known about the visual response in alert rats. Cortical neurons may have visual responses of greater magnitude, be more diverse and be more sensitive to the context in the alert state (Pack et al. 2001, Qin et al. 2008, Niell and Stryker 2010). Two-photon population calcium imaging of the visual cortex in alert rats, where action potential firing in populations of neurons were followed, has shown that the average neuronal action potential firing decreases when anesthesia is induced. The same study reported that even though action potentials firing are temporally structured in both alert and anesthetized state, the cells fire closer in time in the anesthetized state. The study concluded, on basis of these findings, that how neurons fire in

time and space relative to each other in alert state cannot be directly inferred from recordings done in anesthetized animals, even when considering the same population of cells (Greenberg et al. 2008). Studies of hemodynamic responses in visual cortex of mice have shown that the hemodynamic responses are larger and faster in alert mice than those in anesthetized mice. In alert mice the responses peaked earlier and they did also have a faster return to baseline (Pisauro et al. 2013). Differences in responses between simultaneously recorded areas (V1 and prefrontal cortex) have been found, both when they were recorded from in alert state and under anesthesia (Sellers et al. 2013). This indicates that even though there have been previous studies comparing areas (other than V1) in alert and anesthetized state; this cannot be directly applied on V1.

### **1.7 Aim of study**

A previous investigation finds particular preferences for spatial and temporal frequencies for V1 neurons of rats (Girman et al. 1999), however the investigation was performed with anesthetized animals. Anesthesia has a heavy impact on brain functioning and other reports suggests that preferences for spatial and temporal frequencies may vary between alert and anesthetized state (Niell and Stryker 2008, Alitto et al. 2011, Andermann et al. 2011).

The aim of this study is to investigate preferences in spatial and temporal frequencies for single units in primary visual cortex in alert rats, and compare these findings with preferences found for the same units in anesthetized state.

## **2 MATERIALS AND METHODS**

### **2.1 Approvals and research animals**

All experiments were done at the Department of Biosciences (IBV), Faculty of Mathematics and Natural Sciences, University of Oslo, Norway. Experiments and procedures with experimental animals were approved by the Norwegian animal Research Committee (FDU). Housing of animals satisfies regulations set by EU and FDU. As required by the Norwegian Food Safety Authority (Mattilsynet), all involved researchers hold an animal researcher certificate (FELASA C).

Two male Long Evans rats were used. They were 3-4 months old and weighed approximately 500g when they were chronically implanted with microdrives carrying 16 electrodes each into both hemispheres of the primary visual cortex (V1). Electrophysiological recordings of single units were conducted from the same neurons when the animals were alert and anesthetized.

The animals were kept on a 12h light/dark cycle, with light on from 8:00 pm to 8:00 am. Light intensity during the light phase was >100 lux. Rats are nocturnal animals and all recordings were done during the dark phase. The temperature in the animal cabinets was  $21 \pm 0,1^{\circ}\text{C}$ , and air humidity was  $55 \pm 10\%$ . The air in the housing room was replaced 5-20 times per hour. Before surgery, animals were housed 3-4 together in polycarbonate cages (35x55x19cm). In order to minimize the risk of damage to the microdrives after surgery, animals were housed individually in plexiglas cages (35x55x30cm). In both cases the floor was covered with woodchip bedding, and the cage was equipped with plastic toys. Pre-surgery, rats had access to food and water ad libitum while they received 10 food pellets per day after surgery.

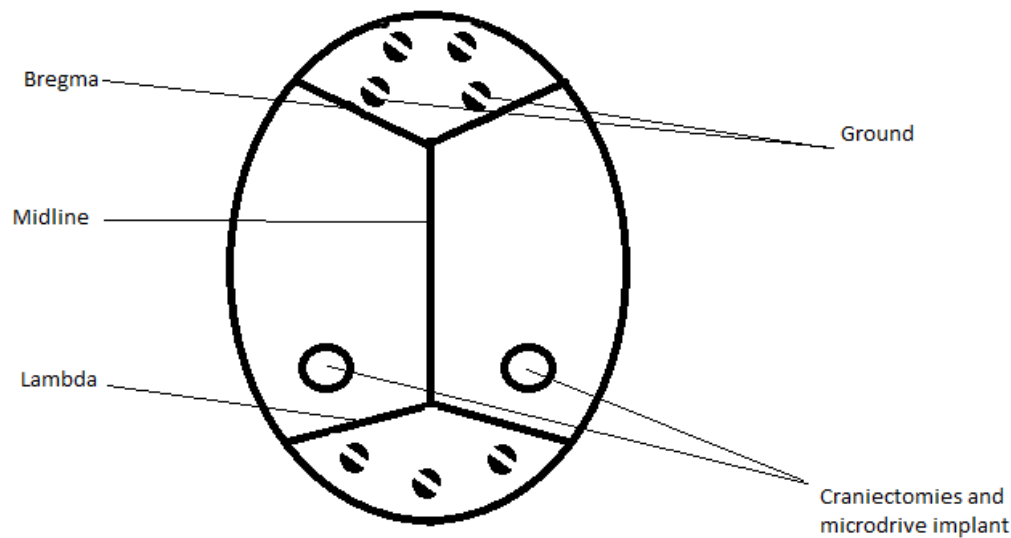
## **2.2 Surgery preparations**

The microdrives that were chronically implanted in V1 in both hemispheres have four tetrodes each, which are made up of four electrodes each. The tetrodes were prepared as described in (Fyhn et al. 2004). Impedance of the tetrodes was reduced from approximately 1500 k $\Omega$  to 120-250 k $\Omega$  by electroplating in platinum solution (Ferguson et al. 2009). The implants with dental cement weighed approximately 5-7g (1-2% of body weight), and did not cause any observable constraints to the animals.

## **2.3 Surgical procedures**

Anesthesia was induced with 3% vaporized Isoflurane, and maintained with a mixture of Ketamine (60 mg/kg) and Medetomidine (0,6 mg/kg) injected intraperitoneally. The level of anesthesia was continuously monitored by observing heart rate, breathing frequency and checking toe pinch reflexes. Additional doses of Ketamine were applied if needed.

The rat's head was shaved between the eyes and back behind the ears. The head was immobilized using a stereotaxic frame (World Precision Instruments Ltd, Hertfordshire, UK) with ear bars in the external auditory meatus. The site of incision was washed with ethanol and iodine solution. A longitudinal incision was made in the skin, and tissue was moved aside using artery clamps. The membranes on top of the skull bone were removed. Seven holes for attachment of jeweler screws were drilled in the skull; four anterior and three posterior (Figure 8). Craniotomies were drilled laterally of midline, one on each side. The craniotomies were drilled 5,0 mm laterally to the midline and 2,6 mm anterior of the transverse sinus on both sides. A small incision in the dura mater was made and the tetrodes were inserted from 4,7 to 5,0 mm lateral to the midline in a 30° degree angle, with the tip pointing in the lateral to medial direction. The tetrode tips were lowered between 350-500 $\mu$ m from the surface (ventrally). To secure the microdrives to the skull, several layers of dental cement was applied.



**Figure 8: Overview of exposed skull.** Four jeweler screws were attached anterior to bregma and three jeweler screws were attached posterior to lambda. The two craniotomies were drilled above visual cortex 5,0mm lateral to the midline on each side, and 2,6mm anterior of sinus.

To minimize the risk of infections, all the surgical equipment was either autoclaved (cotton swabs) or heat sterilized (150°C for 90 minutes).

In order to reduce pain and minimize risk of infection post-surgically, the rats were given Rimadyl (5 mg/kg), Penicillin (13,5 mg/kg) and Convenia (8 mg/kg) for three days following the surgery. The rats were allowed to recover for at least three days after the surgery before experiments began. In order to evaluate if the rat was in pain and needed more than three days of recovery or supplementary doses of analgesia, Langford's table for coding of pain in laboratory mouse was used (Langford et al. 2010). The surgeries were performed by my supervisor, Ida Aasebø.

## **2.4 Experimental protocol**

The electrophysiological recordings were conducted both when the animals were alert and freely behaving, as well as during anesthesia. Recordings during anesthesia were only performed if the same units had been recorded from in alert state.

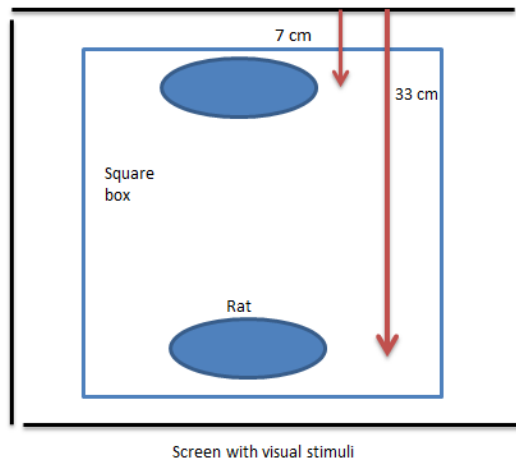
The rats were picked up from their cages and their microdrives were attached to two headstages connected to cables that connect to the recording setup (Axona, UK). In order to continually follow the same units between days, channel 1-16 was always connected to the drive in the left hemisphere, D1, and channels 17-32 was always connected to the drive in the right hemisphere, D2.

### **2.4.1 Visual stimulation**

The rat was placed in a square glass box (27,5x27,5x34,5cm), where it could move freely. Surrounding all the four sides of the glass box were (17") LCD screens (Dell). To ensure that the wires did not bother the rat, they were attached to a counterweight system in the ceiling. Visual stimuli consisting of (sinusoidal) drifting gratings with different spatial and temporal frequencies were presented on the monitors, by custom written Matlab code (Niell and Stryker 2008), based on Psychophysics Toolbox (Brainard 1997). The drifting gratings were presented in 8 different orientations for 12 repetitions (Figure 10B). The duration of each orientation was 0,5 sec. Between each stimuli, a gray screen was presented for 0,3 sec. The screen illumination was set to the lowest possible level (60 – 70 lux), in order to recruit as many visually responsive neurons as possible.



Experimental setup seen from above



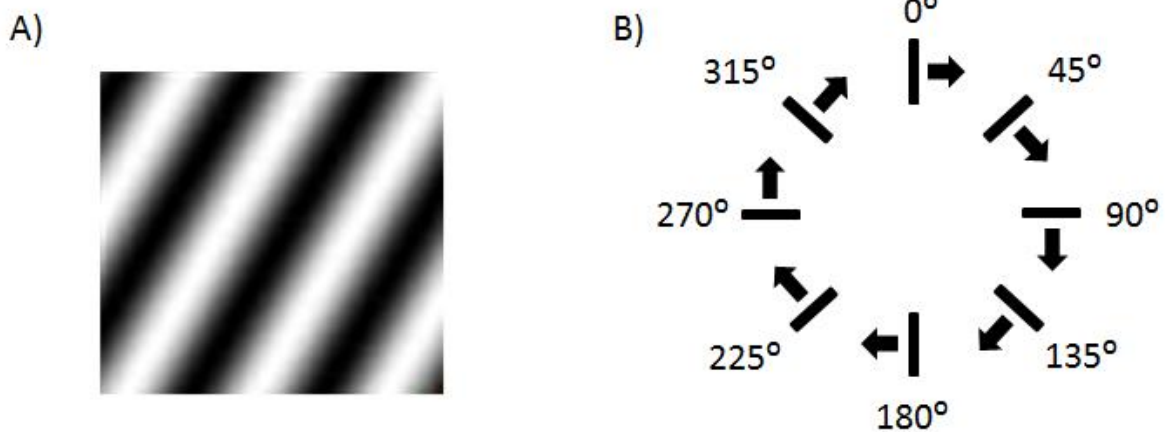
**Figure 9: Experimental setup.** The distance from the screen and the rat was set to the average of the closest possible distance and the longest possible distance between the screen and the rat.

The spatial frequency perceived by the rat, depends on the distance from the stimuli. This distance is set to a constant in the software program. However, since the rat was able to move freely in the square box, the actual distance from the screen varies. The distance was therefore set to the average between the smallest and the biggest distance the rat could possibly be from the screen. For all sessions, the rat's activity level was measured using a tracking diode. Sessions were included only if the rat sat still throughout the whole session.

In each session, a specific temporal or spatial frequency was tested (Table 2). In every experiment, the parameter order was changed randomly and each frequency was tested twice. The ranges of spatial and temporal frequencies are based on the frequencies that produce a variety of responses in mouse and rat (Girman et al. 1999, Niell and Stryker 2008). Whenever temporal frequency was varied, spatial frequency was kept at a constant of 0,08c/d, and when spatial frequency was tested, temporal frequency was kept at a constant of 4 Hz. Niell and Stryker (2008) showed that a temporal frequency of 4 Hz yields an optimal response in most units in mouse visual cortex and Girman and co-workers (1999) found that a spatial frequency of 0,08 c/d is the preferred spatial frequency in V1 for anesthetized rats. This is the reason why these values were used as the constants when changing the other parameters.

**Table 2: Stimulus parameters:** Spatial and temporal frequencies of the drifting gratings presented to the rat.

Spatial frequency	Temporal frequency
0,02 c/d	2 Hz
0,04 c/d	4 Hz
0,08 c/d	8 Hz
0,16 c/d	
0,3 c/d	



**Figure 10: Sinusoidal drifting gratings.** A) Example of a sinusoidal drifting grating. B) The sinusoidal drifting gratings were presented in eight different directions.

The stimulus protocol was repeated in anesthetized rats. Anesthesia was induced with vaporized Isoflurane and when light anesthesia was achieved, 1mg/kg Midazolam was injected subcutaneously. The exact time of injection was noted. In order to keep the pupils fully open during the experiment, atropine (tropicamide) was applied to the eyes. To ensure that the Midazolam was absorbed we waited five minutes from injection before continuing with procedures. The rat was exposed to an initial 3% Isoflurane concentration in order to

easily position the rat in the anesthesia mask. When properly placed in the mask, the Isoflurane level was lowered to 1% which was maintained during the whole experiment. In order to monitor anesthesia level, heart rate and oxygen saturation were monitored with Mousestat (Kent Scientific, USA) during the course of the experiment.

#### **2.4.2 Electrophysiological recording**

If no spiking activity was picked up from a unit, the tetrode was lowered by turning a pin on the microdrive. Each round equals 200 $\mu$ m, and the tetrode was lowered 50 $\mu$ m to 100 $\mu$ m at the time.

The units were recorded with daqUSB, a system provides by Axona (Herts, UK). Multichannel head stages were couples to the chronically implanted microdrives. Each microdrive has 16 electrodes. Through a lightweight multi-wire cable, the head stages were coupled to a preamplifier, which was coupled to the 32-channel recording unit. In all experiments, both analogue and digital amplifiers (Axona, Herts, UK) were used. When the signal amplitudes exceeded 4-5 times the noise level, the data collection started. Signals were amplified 6000-12000 times and band-pass filtered between 0,8 and 6,7 kHz. Spikes were stored to disk at 48 kHz (50 samples per waveform, 8 bits/sample) with a 32-bit time stamp (clock rate at 96 kHz). Spikes above threshold of about 80  $\mu$ V were time-stamped and digitized at 32 kHz for 1 ms, and saved to the hard-drive for offline analysis. In each hemisphere one of the 16 channels was used to record EEG. These signals were amplified 5000 times, lowpass-filtered at 500 Hz and sampled at 4,8 kHz (16 bits/sample). To allow synchronized time-stamping of stimuli and spikes, the computer used to generate visual stimuli were connected to the digital input on the Axona recording setup.

## **2.5 Histology**

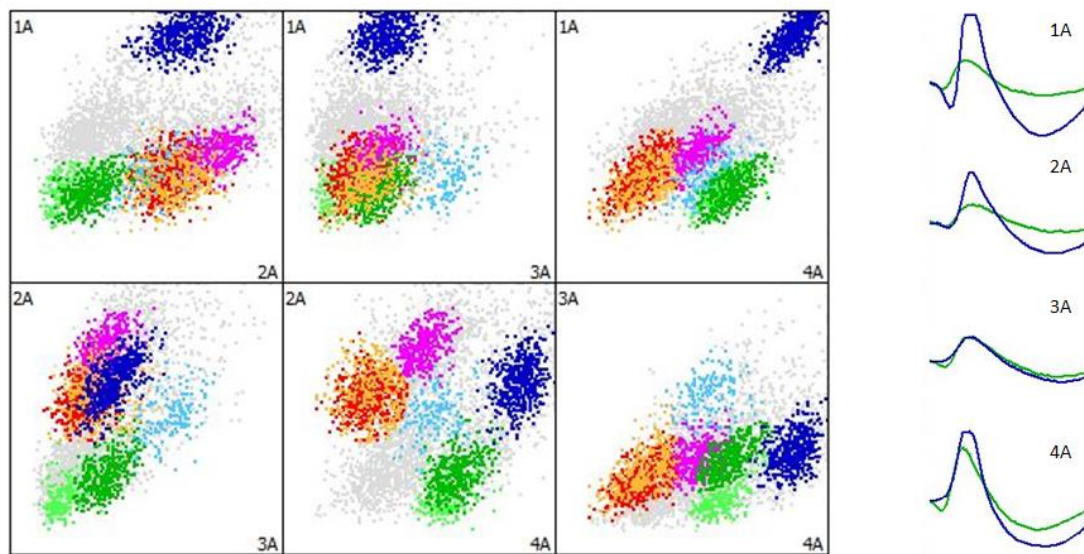
After all experiments the rats were euthanized with an overdose of pentobarbital and intracardially perfused with 0,9% saline and 4% formaldehyde for histological verification of tetrode position in the tissue. Following perfusion the brains were left in 4% formaldehyde overnight and cryo-protected with 30% sucrose in PBS for 72 hours. The brains were subsequently sectioned on a cryostat in 40µm sections. Sections were stained for Nissl bodies with Cresyl Violet (Sigma-Aldrich) in order to identify the anatomical area of the tetrode tracks. A Zeiss microscope (Axioplan 2) with Axiovision software (MosaiX stitching) and AxioCam HRZ camera was used for the histological photos.

Due to fixation and freezing of the brain, the tissue normally shrinks <20%. This was accounted for when investigating the depth of the tetrode tracks.

Superfrost slides with sections of brain tissue were treated with chloroform/ethanol overnight, and then they were hydrated in distilled water and immersed in Cresyl Violet solution. To dehydrate the sections, ethanol and xylene were added. Cover slips were mounted with Entellan (Merck Millipore, Darmstadt, Germany) before they were left to dry overnight in a fume hood. Detailed protocol for Nissl staining is found in the Appendix. The histological procedures were performed by my supervisor, Ida Aasebø.

## **2.6 Spike sorting and data analysis**

In order to separate the different units in the recorded electrophysiology data, graphical cluster-cutting software was used (Tint: Axona Ltd, Herts, UK). The cluster-cutting method is a way of separating individual single units from each tetrode using two dimensional scatter plots based on the waveform amplitudes of individual spikes. Spike amplitudes are shown as points in the plot, and spikes from a single unit tend to cluster together (Figure 11). These can be manually cut by enclosing them in a boundary and color-code the isolated clusters. In this process it is important to make sure that the clusters are separated from noise. As an additional tool to ensure that the boundaries were correct, temporal autocorrelation of spikes within the cluster were used. If the cluster contained several spikes that fired within the 2 ms refractory period of a neuron, it was presumed that the cluster contained spikes from a different unit (Harris et al. 2000), and the isolation procedure was repeated.



**Figure 11: Manual spike sorting.** 1A-4A are the four electrodes on a tetrode. The amplitudes for the single spikes recorded on the electrodes are plotted in scatter plot (left). Spikes belonging to the same single unit tend to cluster together. These clusters are enclosed and given color. The average waveforms for spikes chosen to the same cluster are shown to the right.

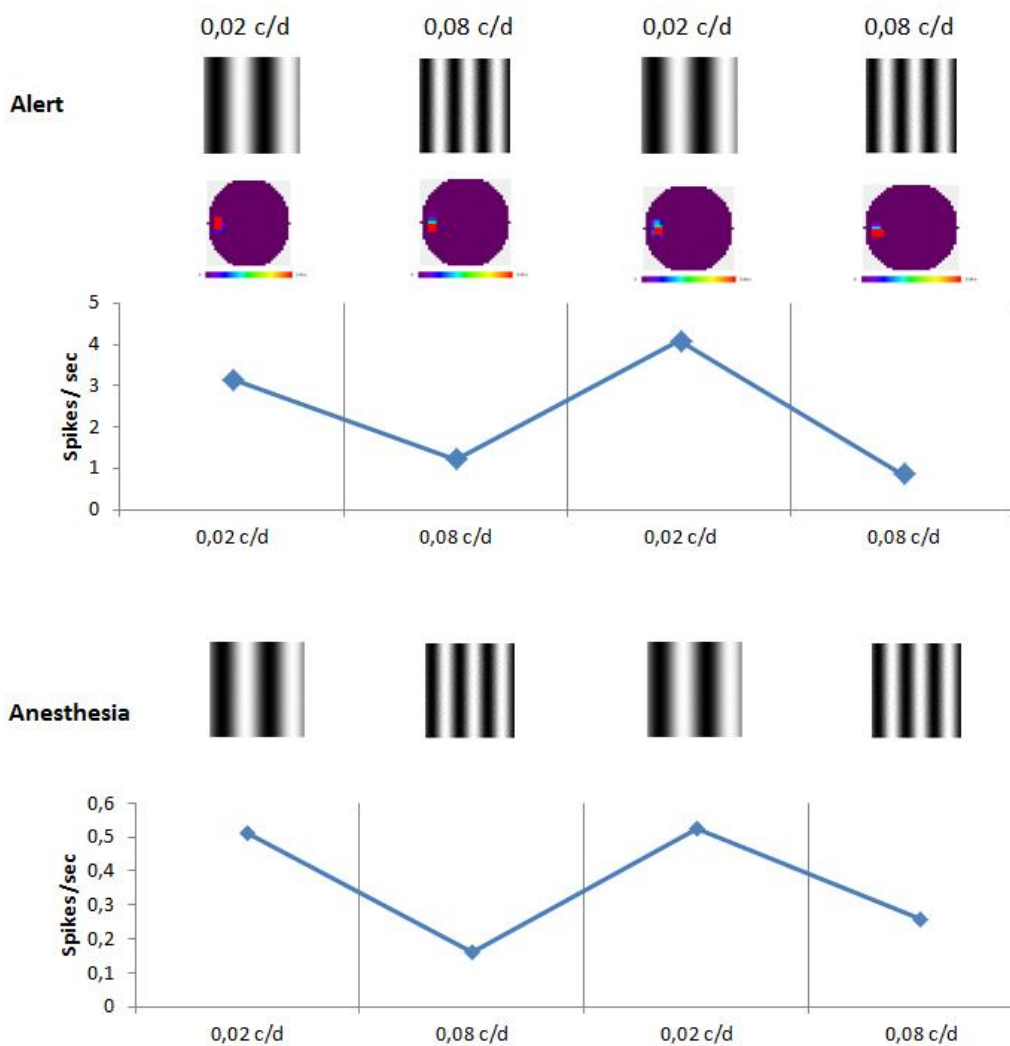
## 2.7 Statistical analysis

All statistical analysis was performed in Microsoft Excel 2010 or GraphPad Prism 6. If the data failed the D'Agostino-Pearson omnibus normality test, a nonparametric Wilcoxon Signed Ranks test was used. This was used to analyze reduction in firing rate between alert state and anesthesia and the test for differences between the sessions. When comparing normalized ratios of firing rate for spatial frequencies in alert state, spatial frequencies in anesthetized state, temporal frequencies in alert state and temporal frequencies in anesthetized state, Two way repeated measures ANOVA test have been used because more than two groups were compared. Further, to investigate differences in selectivity for spatial frequencies in alert state, spatial frequencies in anesthesia, temporal frequencies in alert state, temporal frequencies in anesthesia, a non-parametric Friedmans test was performed, due to the non-normality of the data. The statistical analyses were conducted by my supervisor Ida Aasebø.

### 3 RESULTS

After the cluster-cutting procedure, a total of 25 single units were included for further analysis. The remaining units were excluded in the cutting process, either because they disappeared during anesthesia, were difficult to isolate from the other units or could not be isolated from the background noise. An average between first and second recording for a frequency and the difference between the two was calculated. These values were used in the following analyses, if not otherwise is mentioned. All included units are presented in Appendix.

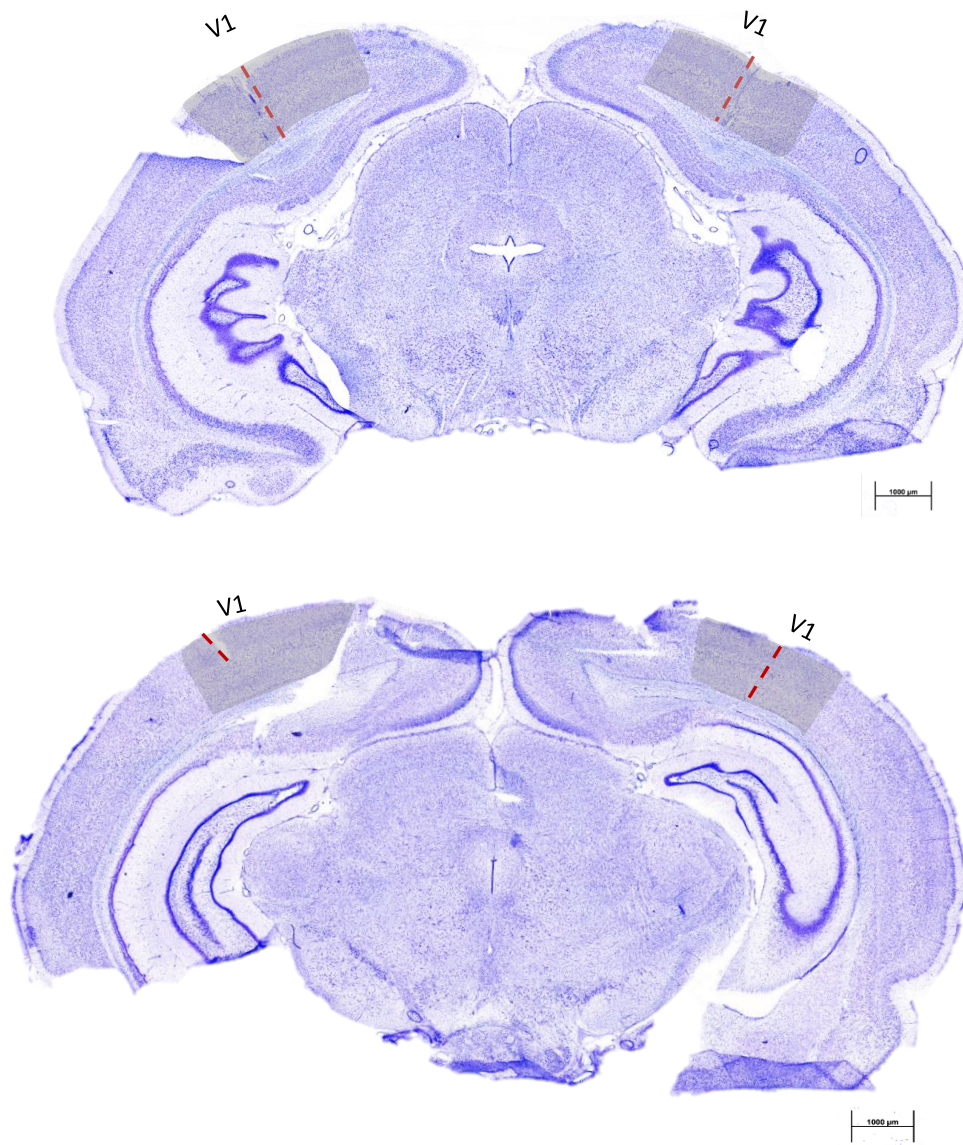
#### Example single unit:



**Figure 12: Experimental set-up.** Upper panel: Example of the experimental setup and the responses from one unit to two spatial frequencies in alert state (unit 8). All frequencies were presented twice and in random order. Color coded map of the rat's position during the experiment showing in warm colors (red) where the rat spent most time (purple: no tracked positions during recording). The plots indicate the typical behavior in all trials; the rat was mostly sitting still. Bottom panel: Same procedure during anesthesia. Note a clear preference for 0,02 c/d for this unit.

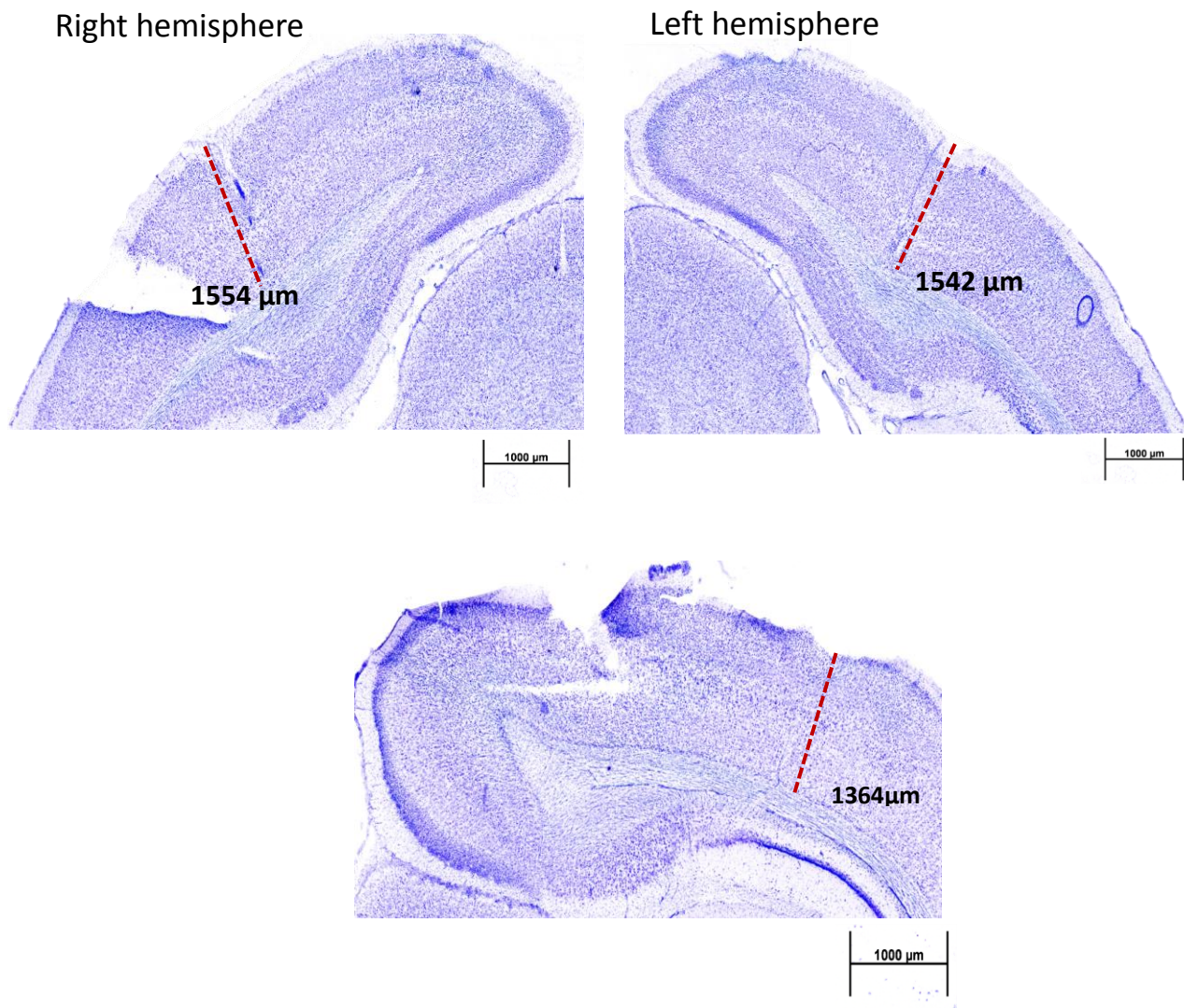
### 3.1 Histology

Coronal sections, stained for Nissl bodies, show that the tetrode tracks in the two brains were positioned in the primary visual cortex (Figure 13). The primary visual cortex can be identified by an enhanced cell density in cortical layer 4 and 6, relative to adjacent areas. The length of the tetrode tracks in Figure 14 is 1554 $\mu$ m, 1542 $\mu$ m and 1364 $\mu$ m. The length of the fourth tetrode track cannot be reported due to tissue damage during histology preparations. However all tracks were verified to be localized in V1. The depth of the tetrode tracks indicate that the last recording sessions were from layer 5. The three experiments reported in this project were done in Layer 2/3 to 4 and Layer 5



**Figure 13:** Coronal section with tetrode tracks in primary visual cortex. Upper figure is section from rat 1446. Lower figure is section from rat 1444. Primary visual cortex in gray, tetrode tracks marked with dotted red line.

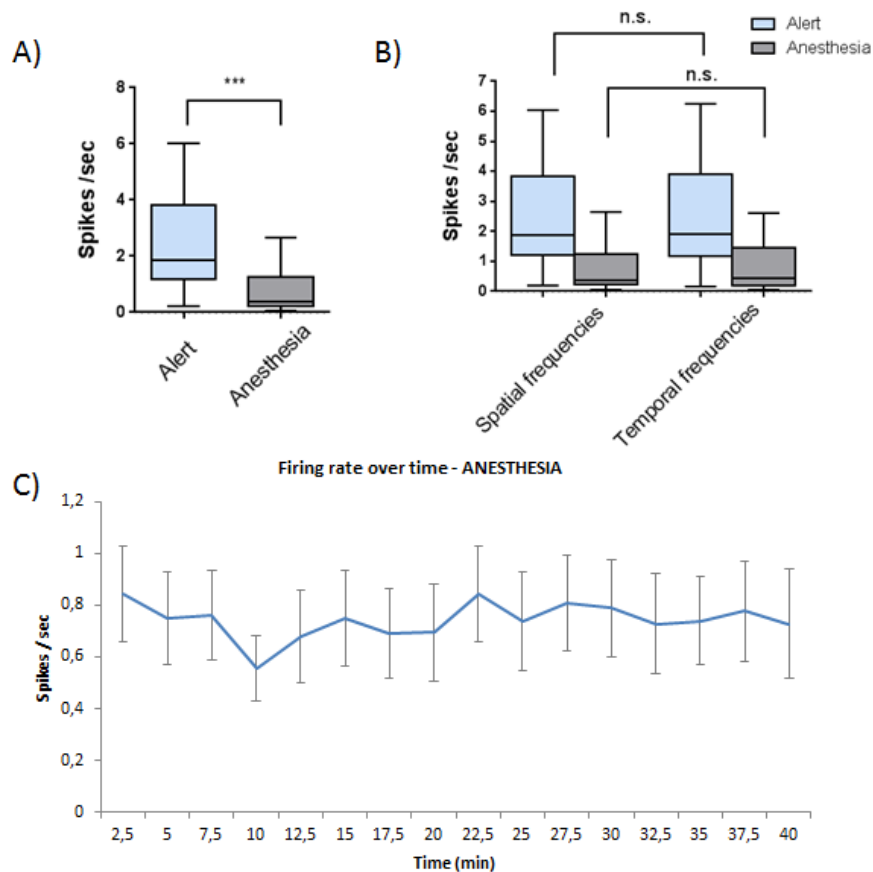




**Figure 14: Depth of tetrode tracks.** Upper figure is sections from rat 1446. Lower figure is section from rat 1444. Right hemisphere is not shown due to tissue damage during staining.



### 3.2 Effect of anesthesia

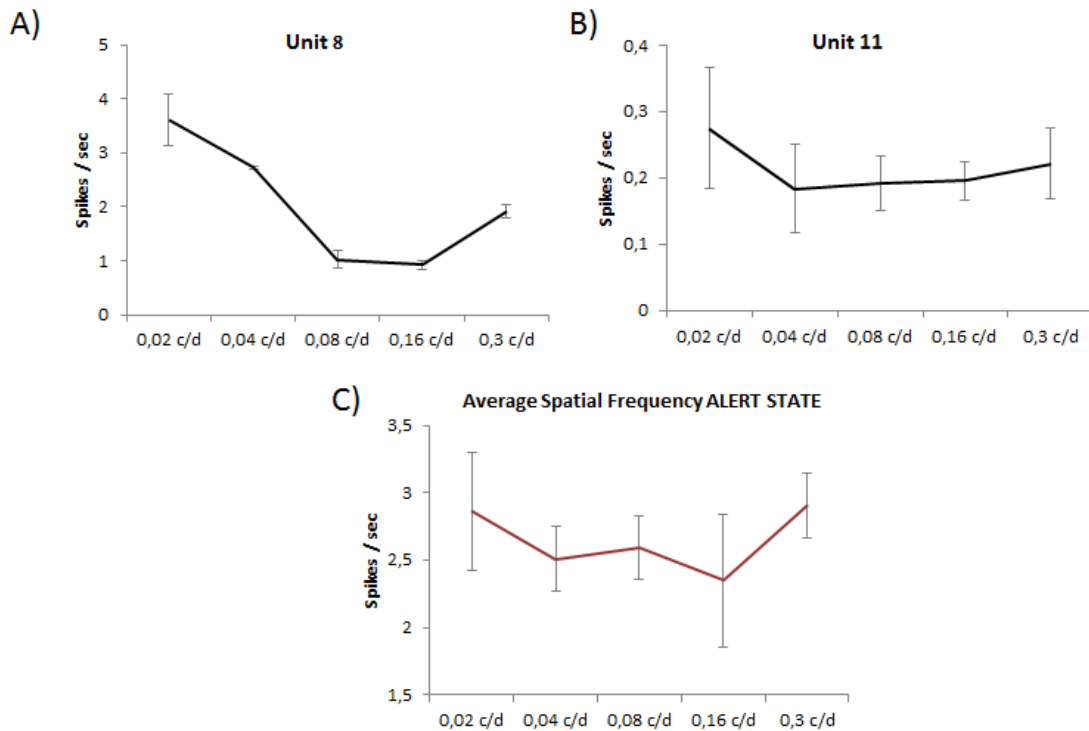


**Figure 15: Anesthesia leads to a reduced average firing rate in visual cortex.** A) The reduction in firing rate from alert state to anesthesia was 53%. (N=25  $p < 0.0001$ , Wilcoxon Signed Ranks test). B) The average firing rates were similar when the stimuli of spatial and temporal frequencies were compared in both states (Non-significant Wilcoxon Signed Ranks test). Boxplots indicate the median and the upper and lower percentile, whiskers indicate Tukey interquartile range. C) Firing rates of single units during anesthesia were stable over time (n=25) Mean  $\pm$  SEM (standard error of the mean).

A comparison between average firing rate for all single units in alert and anesthetized state, shows a significant reduction of 53% during anesthesia (n=25,  $p < 0.0001$ ). There is no difference in average firing rates between spatial and temporal frequencies in the different states. The reduction in firing rate is in accordance with previous findings in the research group (Aasebø et al. 2013).

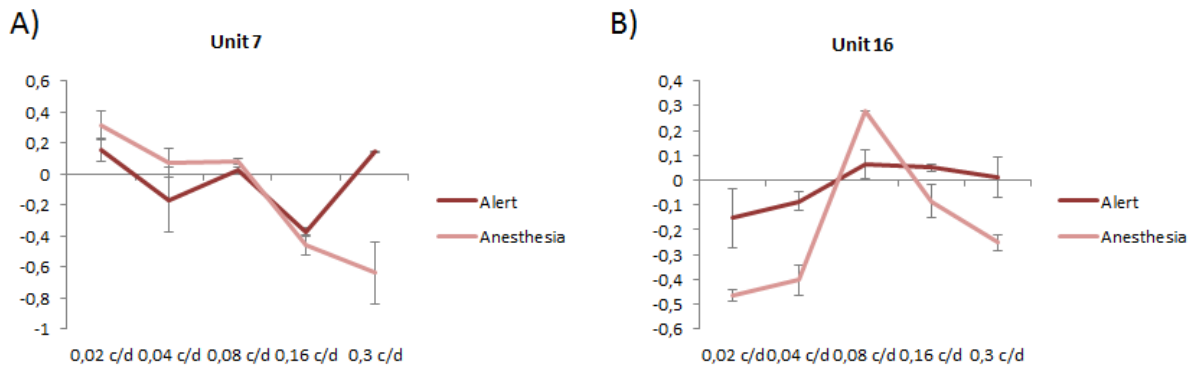
Although there is a significant reduction in firing rate when anesthesia is induced, the firing rate in anesthesia is stable during the entire course (40 min) of the recording.

### 3.3 Spatial Frequency Preference



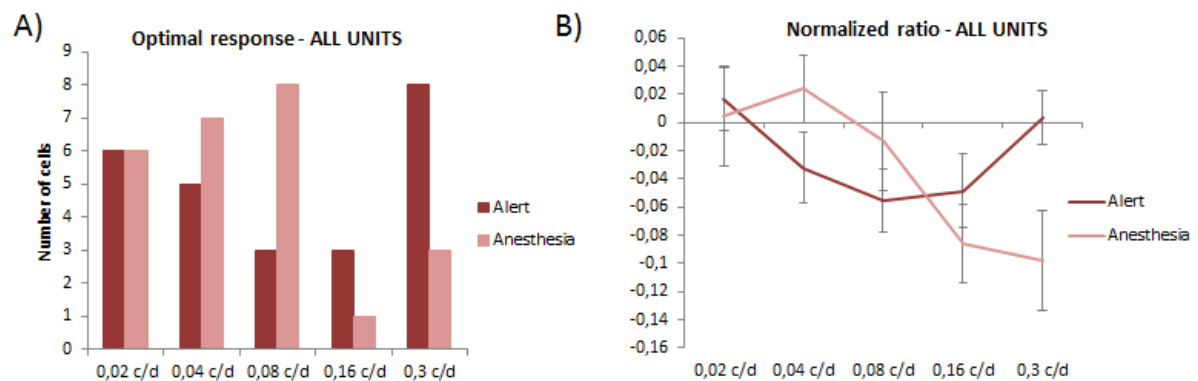
**Figure 16: Spatial frequency preference varies between units.** A) A unit with a clear preference for a spatial preference in alert state. Small error bars indicate a small difference between the two recording sessions. B) A unit with no specific preference in alert state, the difference between the two sessions is also big. C) Average firing rate for all single units for all the spatial frequencies presented.

The recorded units vary in their preference for the different spatial frequencies. Some have a clear preference for one spatial frequency (Figure 16A); others do not show such preferences (Figure 16B). The difference between two sessions measuring the same frequency also varies. Thus average firing rate for all units over all spatial frequencies, show no clear preference. Since each unit has quite a different firing rate, any change occurring for a particular unit would be difficult to identify when looking at mean firing rates alone (Figure 16C).



**Figure 17:** Example of single units with different responses for the different spatial frequencies between alert and anesthetized states. A) Unit 7, normalized ratio of mean firing rate in alert state (red) and anesthesia (pink). B) Unit 16, normalized ratio of mean firing rate in alert and anesthetized states.

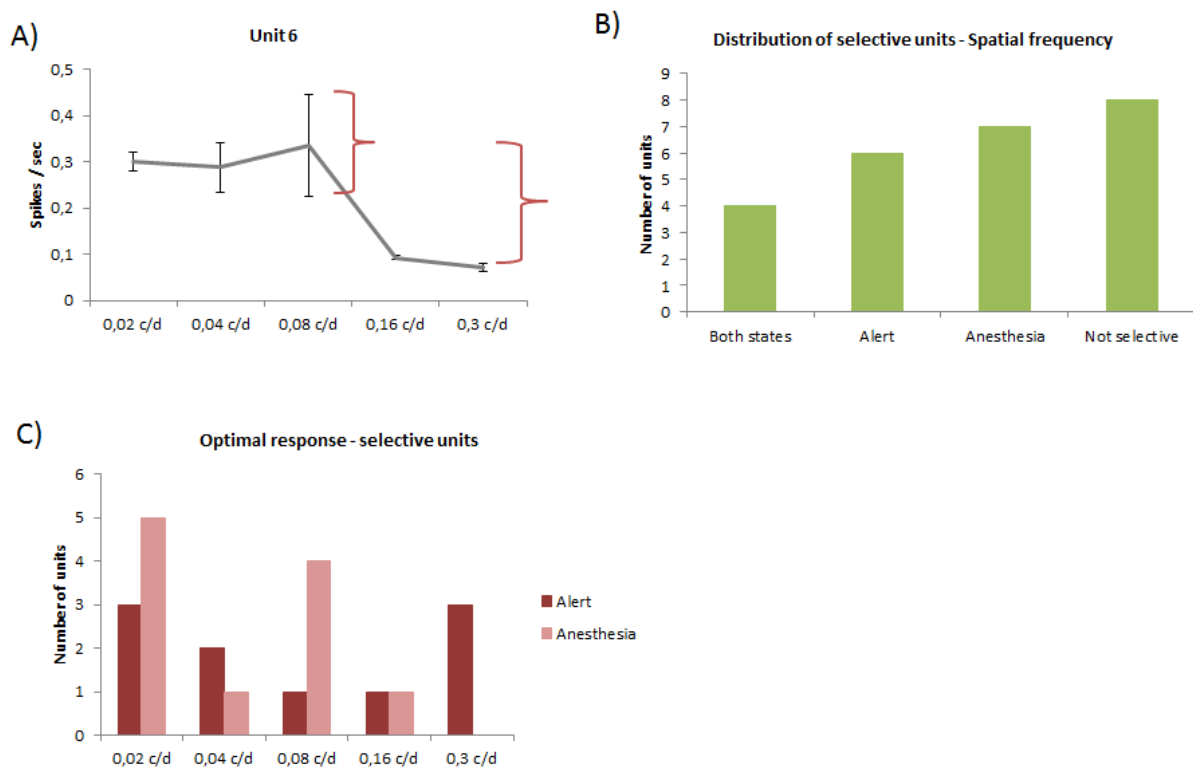
Not only do some of the units have a preference for a spatial frequency, but some of the units have a preference for different spatial frequencies in alert state versus anesthetized state (Figure 17A and B).



**Figure 18:** The units' preferred spatial frequency. A) Optimal response for all units in alert state (red) and anesthesia (pink). B) Normalized values for the units' mean firing rate in alert (red) and anesthetized (pink) states. Mean  $\pm$  SEM. Non significant Two way ANOVA.

Based on the units' optimal response (the frequency that gives the highest mean firing rate), more units have a preference for higher spatial frequencies in alert state than they have under anesthesia (Figure 18A). In other words, animals that are alert tend to respond more strongly to higher spatial frequencies. The most frequent optimal response to spatial

frequency in alert rats is for 0,3 c/d, whereas in anesthesia it is 0,08c/d. Since the units have very different firing rates, we calculated a normalized ratio of the mean firing rate for each unit where the firing rate for a specific stimulus was divided by the unit's average firing rate for all frequencies. Although not significant, the normalized ratio of mean firing rate for all units shows the same tendencies as the units' optimal response. In the alert state it seems to be a bimodal shape and they respond more strongly to higher spatial frequencies than in the anesthetized state.

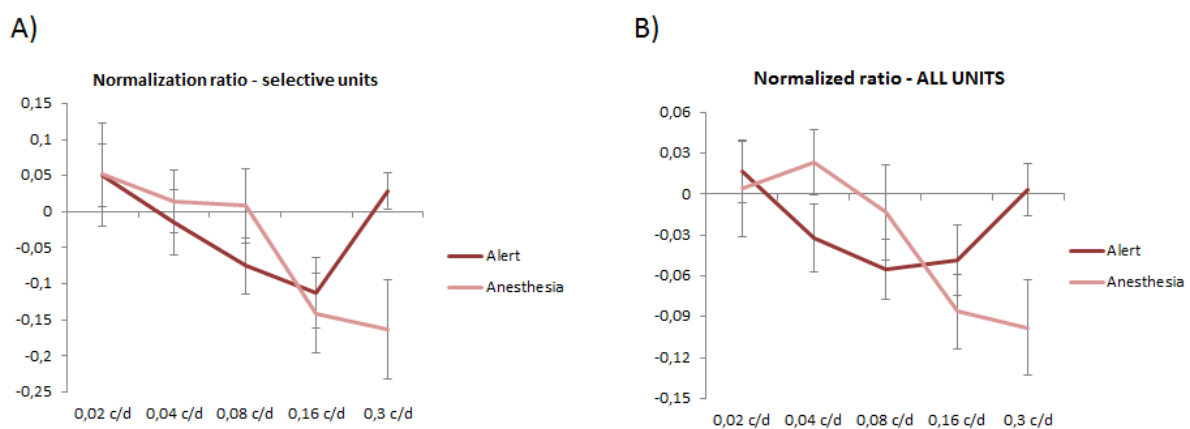


**Figure 19: The units were categorized into selective and non-selective for spatial frequencies.** A) Illustration of selection criterion. For a unit to be categorized as selective, the difference between frequencies must be bigger than the naturally occurring differences in firing rates within one frequency. B) Distribution of selective units between selective only in alert state, selective only in anesthesia, selective in both states and not selective. C) Preferred spatial frequency (optimal response) for units that are categorized as selective units in either alert state, anesthesia or both states (n alert = 10, n anesthesia = 11).

When analyzing every single unit, it was apparent that some units clearly had a preferred spatial frequency where the firing rate increased, whereas others did not. Therefore we wanted to analyze only the single units with a clear preference for a spatial frequency. We called the single units that had a preference; selective units. In order to categorize selective units from the non-selective units, we compared the difference in firing rate between the

highest response and the lowest response, with the biggest difference seen between two recording sessions with the same spatial frequency. A single unit was considered selective if the difference in firing rate between sessions with the same frequencies was lower than the difference between frequencies (Figure 19A).

Using this criterion, four single units are selective in both the alert and anesthetized state, six are selective only in the alert state, seven are selective only in the anesthetized state and eight single units are not selective in either of the states. This means that 17 of the 25 single units (68%) are considered selective for spatial frequencies in either alert or anesthetized state, or both. When comparing the optimal response for selective units, the same trend is obvious as when comparing the optimal response for all units. In alert state there is an equal distribution of single units preferring 0,02c/d and 0,3c/d, whereas in anesthesia no single unit prefer the highest spatial frequency (0,3 c/d). Most single units in anesthesia prefer 0,02c/d.

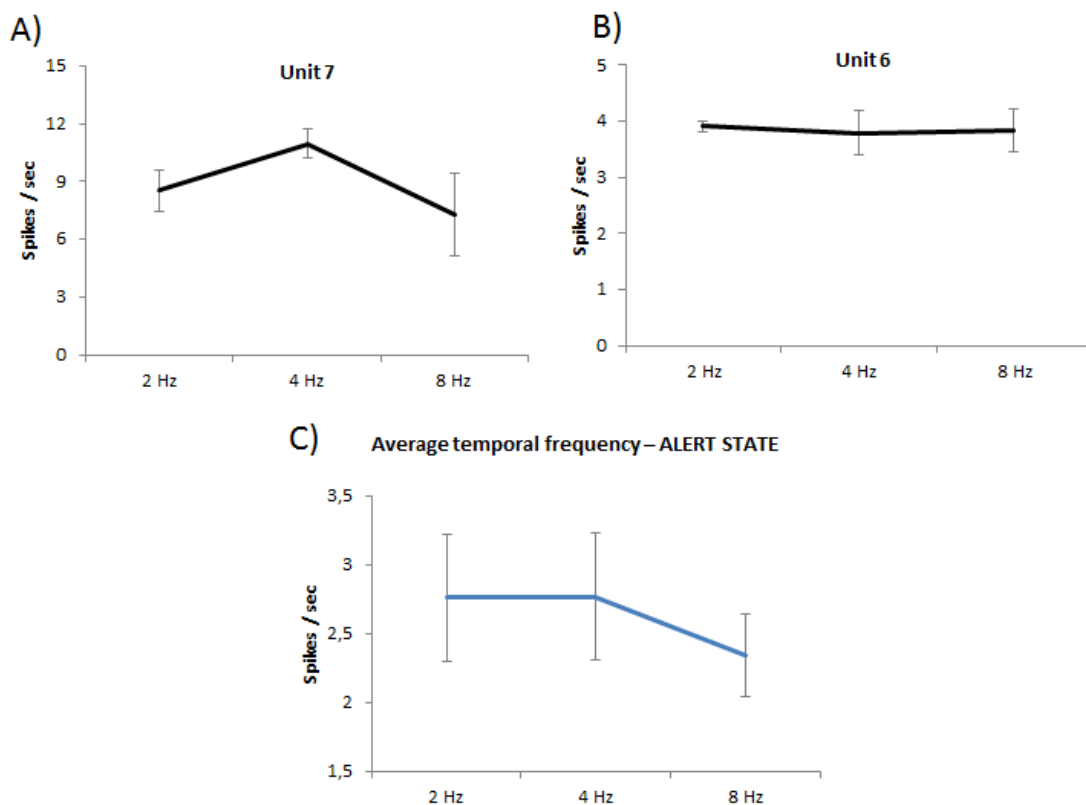


**Figure 20: Comparison of responses from selective units to all units.** A) Normalized ratio of mean firing rate for selective units. B) Normalized ratio of mean firing rate for all units, for comparison.

The normalization ratio of mean firing rate for the selective units, show a similar tendency as the whole population of units. The preference for 0,02c/d for selective units under anesthesia is still present, compared to a preference for 0,04 c/d for all units, meaning that the average firing rate for all units have a higher relative increase in firing rate at 0,04c/d than the selective units. Single units in alert state show a much stronger preference for 0,3

c/d than units under anesthesia. Units under anesthesia show a stronger preference for 0,08c/d than they do in alert state. The graph showing normalization ratio in alert state includes ten units, whereas the graph showing normalization ratio in anesthesia include 11 units. Hence the four units which are selective in both alert state and anesthesia are included in both graphs.

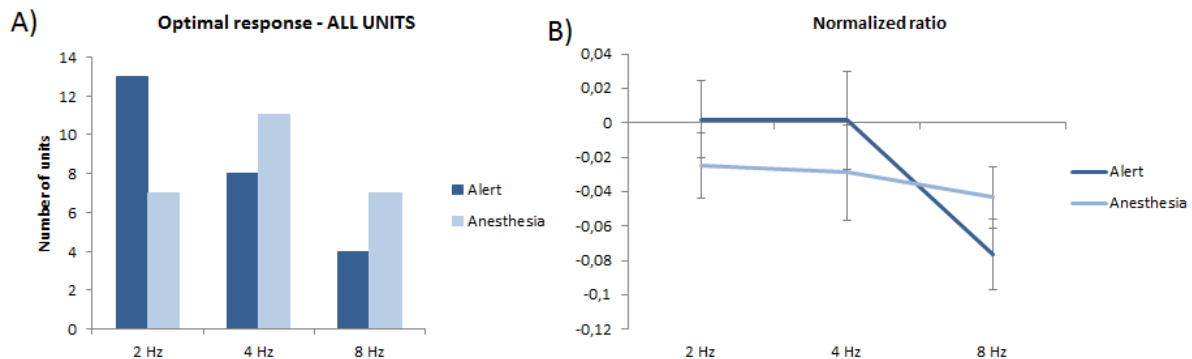
### 3.4 Temporal Frequency Preference



**Figure 21: Temporal frequency preference varies between units.** A) A unit with clear preference for a temporal frequency in alert state. B) A unit with no clear preference for a temporal frequency in alert state. C) Average mean firing rate for all units over all frequencies.

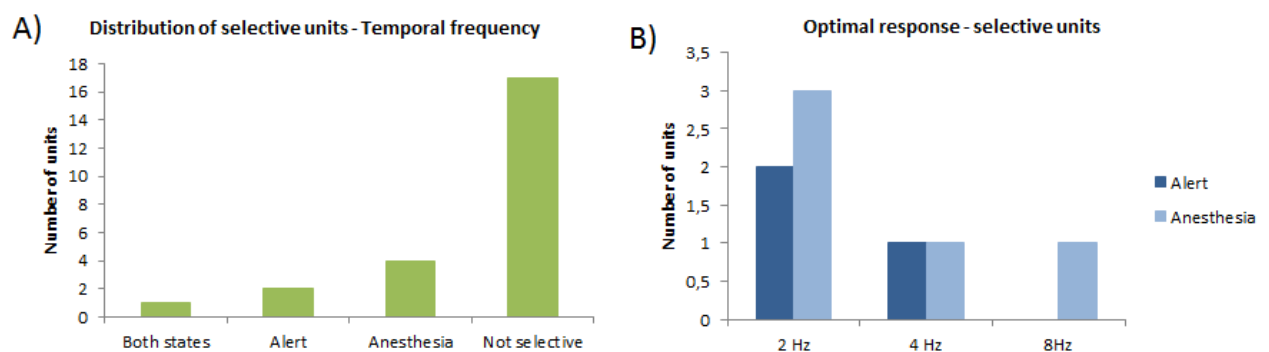
Next, we examined how single units are modulated by temporal frequency of the stimulation. As for spatial frequencies, a comparison between average mean firing rates for

all single units does not show any clear preferred temporal frequency in alert state (Figure 21C), even though there are single units that have a preference (Figure 21A).



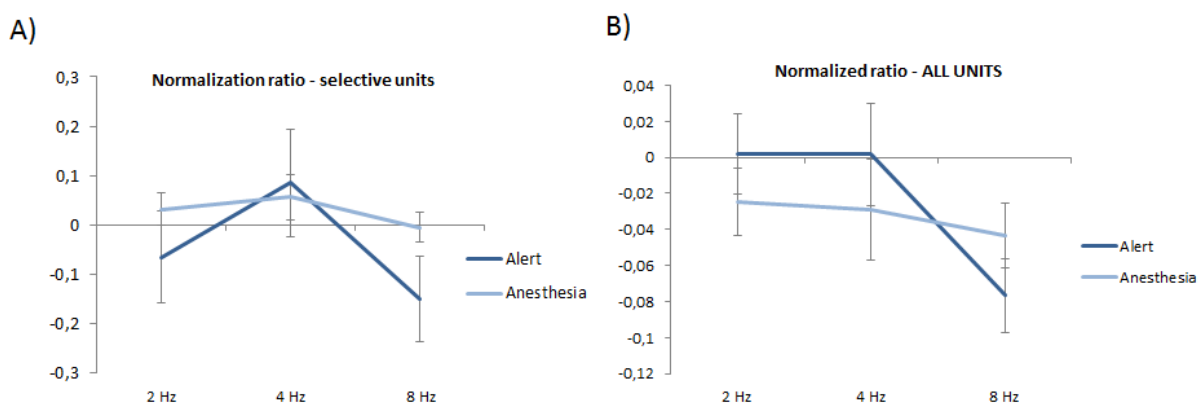
**Figure 22: The units' preferred temporal frequency.** A) Optimal response for all units in alert state (blue) and in anesthesia (light blue). B) Normalized values of mean firing rate for all units in alert (blue) and anesthetized (light blue) states. Mean  $\pm$  SEM. Non significant Two way ANOVA.

When analyzing the number of units based on their optimal responses, a tendency for preferring lower temporal frequencies in the alert state is apparent. The preferred temporal frequency for all units in alert state is 2 Hz, compared to 4 Hz in the anesthetized state (Figure 22A). Normalization ratio of mean firing rate was calculated to compare the units with high firing rate to those with low firing rate. This does not show a relative increase in firing rate for 4 Hz in anesthesia even though most units prefer this frequency.



**Figure 23: The units were categorized into selective and non-selective for temporal frequencies.** A) Distribution of units. B) Preferred temporal frequencies for units that are categorized as selective units in either anesthesia, alert or both states (n alert = 3, n anesthesia = 5).

Using the same selectivity criterion as for spatial frequency, only one unit is selective for a temporal frequency in both alert and anesthetized state, two units are selective only in alert state, four are selective only in anesthetized state and 18 units are not selective at all. This means that three units are selective in alert state, and five units are selective in anesthesia (Figure 23A). Altogether, 7 of the 25 units (28%) meet the selection criterion. For the three units selective in alert state, the optimal response for temporal frequency is 2 Hz. The optimal response for the five units selective in anesthesia is also 2 Hz (Figure 23B).



**Figure 24: Normalized ratio.** A) Normalized ratio of mean firing rate for the selective units. B) Normalized ratio of mean firing rate for all units, for comparison.

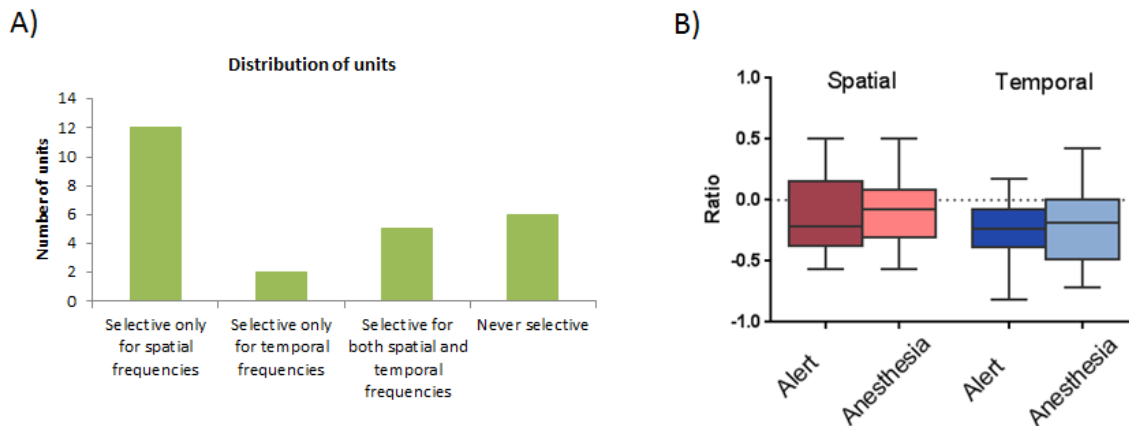
The normalization ratio of mean firing rate for the selective units in alert state shows that these units have a relative increase in firing rate for 4 Hz. Selective units in anesthesia do not show any clear relative increase in firing rate for any of the temporal frequencies. In alert state the selective units do not respond as strongly to 2 Hz as all unit do.

Due to a MatLab error, one recording session for 4 Hz in alert state (Experiment nr. 2 including 6 units) and one session for 8 Hz in anesthesia (Experiment nr. 3 including 12 units) could not be analyzed. Hence, for these units at these two frequencies, only one recording session was analyzed in MatLab. An average value between the two recording sessions measuring the same frequency could not be calculated. In these two cases only the mean firing rate for the units from one session were used when calculating average normalized ratio for the units (see units in Appendix).



### 3.5 Relationship between selectivity for spatial and temporal frequencies

More units meet the selection criterion for spatial frequencies than temporal frequencies ( $n$ , spat = 18;  $n$ , temp = 7). 48% of the units meet the criterion only for spatial frequencies, 8% meet the criterion only for temporal frequencies, 20% of the units meet the criterion for both spatial- and temporal frequencies and 24% of the units do never meet the criterion.



**Figure 25: Relationship between spatial and temporal frequencies for selective units.** A) Distribution of selective units. B) Normalized ratio for all units. (Non significant Friedmans test,  $n=25$ )

To test whether there is a difference in selectivity between the spatial and temporal frequencies, the selectivity ratio for the different groups were tested with Friedmans test (Figure 25B). The calculation of selective ratio followed  $(F1-F2)/(F1+F2)$ , where  $F1$  is the highest response-lowest response, and  $F2$  is the highest observed difference between sessions measuring the same frequency. No significant differences are observed, however a trend towards more selectivity for spatial frequencies is present.

## 4 DISCUSSION

### 4.1 Methodological considerations

The use of chronically implanted multi-electrodes to record activity from neuron populations is a method that yields many advantages, compared to acute single electrodes. First, when the multi-electrodes are chronically implanted, recordings of single units can be conducted in the alert and freely moving animal as well as in anesthesia. This makes it possible to follow the very same neurons from alert state to anesthesia and enhances the methodological strength of the observed differences. In comparison, several other scientists have conducted their experiments on two different populations (Niell and Stryker 2010, Sellers et al. 2013), a method that to a larger degree is dependent on equal sampling of the two populations and no confounding factors in the experimental setup. When each unit can serve as its own control, as with chronic single unit recordings, the occurrence of such confounding factors are minimized.

Electrodes in a tetrode configuration can simultaneously detect signals from a neuron on all four channels. Since the amplitude varies as a function of the distance from the electrode, different neurons will have different spike waveform on the different channels. This makes it possible to sort spikes belonging to different neurons and thereby isolate the units from each other. The microdrive has four tetrodes which enables simultaneous recordings from a population of neurons (Buzsaki 2004). The tetrodes can be moved deeper into the brain while the animal is alert. By lowering the tetrodes, sampling can be done in different brain regions or cell layers of the cortex. In this way large datasets are achieved from each animal. A disadvantage is that chronic electrophysiology recordings are technically challenging to perform and time consuming.

Sorting of spikes into separate single units is done manually and is therefore affected by some degree of subjective decisions (Harris et al. 2000). To reduce this bias, all spike sorting were done before further analysis of the units. Spike clusters that were difficult to separate from other units due to large overlap in the clusters or the noise were excluded. Units were also excluded if the lag time in autocorrelograms was  $< 2$  ms (Harris et al. 2000).

Tracking data show that the rat often sat in the same position in all sessions. The fact that the perceived spatial frequency could differ between sessions even though the same

frequency was tested could give rise to a great difference. This could be a drawback with the method. However, the collected data did not show a greater difference between sessions presenting the same spatial frequency, than for temporal frequencies (see Figure in supplementary analysis in Appendix).

## **4.2 Discussion of main findings**

The aim of this study was to investigate if neurons in primary visual cortex show different preferences for stimuli with different spatial and temporal frequency in alert versus anesthetized rat. The main finding is that in the alert state neurons seem to respond more strongly to stimuli with high spatial frequency, while neurons prefer lower spatial frequencies in the anesthetized state. Although this finding is not significant in the total population, the tendency is clear for many neurons. Neurons in alert rats appear to have a higher preference for 0,3 c/d than anesthetized rats. Neurons in alert rats seem to have a lower preference for 0,08 c/d than in anesthetized rats. I did not detect any systemic difference in the responses to stimuli with different temporal frequency in the alert and anesthetized state. The preference for stimuli with different temporal frequencies varied when conducting the different analyses.

Although my project only includes data from a modest number of neurons from two animals, the data are in line with previous findings from anesthetized animals and therefore I find reason to suggest that the novel data from the alert rat is representative.

### **4.2.1 Anesthesia**

My results show a 53% reduction in firing rate when anesthesia is induced. Previous studies show similar effects of anesthesia on firing rates (Alitto et al. 2011, Vizuite et al. 2012, Aasebø et al. 2013). Isoflurane and Midazolam are believed to act as agonists on GABA<sub>A</sub>-receptors and therefore give an increased neural inhibition. The increased inhibition is probably the reason for reduction in firing rates. This could be a specific effect of GABA<sub>A</sub> agonists since it has not been reported a similar decrease in firing rates when using NMDA antagonists, such as for instance Ketamine (Aasebø et al. 2013).

When anesthesia is induced, the firing rate is relatively stable, which is essential prerequisite for the comparisons between firing rates in anesthesia done in this project. If there is a preferred frequency in one recording session it can be compared to the response from other frequencies.

#### **4.2.2 Spatial Frequency**

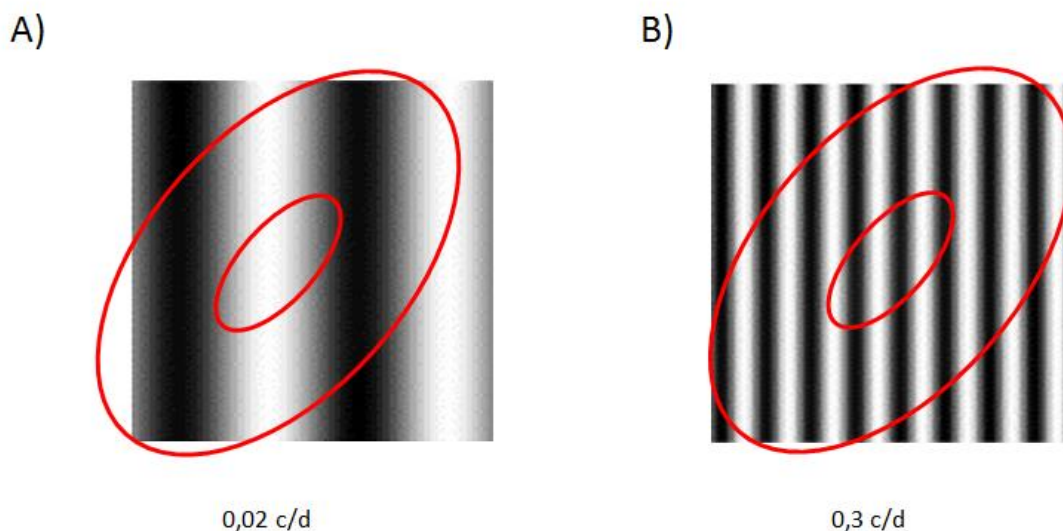
The spiking activity of the neurons recorded in this project show that most neurons have an optimal response to a spatial frequency of 0,3 c/degree in alert state (Figure 18A). In anesthesia the same neurons show an optimal response to a lower spatial frequency of 0,08 c/degree. These results are in line with previous studies of anesthetized rats and mice (Girman et al. 1999, Niell and Stryker 2008). Girman and colleagues (1999) have found that rat V1 neurons, in anesthesia, are sensitive to spatial frequencies up to 1,2 c/d, but most neurons had an optimal response at 0,08c/d. In mouse V1, the maximum response at the optimal orientation is 0,04 c/d and for oriented simple cells 0,08 c/d (Niell and Stryker 2008). In this project we did not distinguish between simple and complex cells, but complex cells are the most common cell type (Palmer 1999), so the neurons included in this project are most likely complex cells. Most of the included neurons are not very orientation selective, so the preference at optimal orientation was not investigated. The fact that we have found the same preferred frequency in anesthetized rats as Girman and colleagues (1999), strengthens the validity of the different findings in the alert animal, since the same neurons are followed from state to state.

A criterion to categorize the selective neurons was introduced in the analysis, as an alternative to the optimal response method. The comparison between these neurons' optimal response also shows the same tendencies, especially for a preferred spatial frequency of 0,3c/d in alert state. No selective neuron had an optimal response for 0,3 c/d in anesthesia. In anesthesia five neurons prefer 0,02 c/d and four neurons prefer 0,08 c/d, meaning that the preference for these two frequencies are quite similar. On the other hand, the comparison of normalized ratio of mean firing rate for selective neurons do show a clear preference for 0,02 c/d in anesthetized state. In earlier studies only the optimal response for all neurons was used (Girman et al. 1999, Niell and Stryker 2008). The optimal response for all neurons is therefore more usable in comparisons of previous results. Based on these

findings, it seems like alert rats can follow higher spatial frequencies than anesthetized rats. To the best of my knowledge, this is the first study to show this. With this in mind, it is possible that rats may be able to detect more detailed structures than one would believe from experiments done on the anesthetized animal.

In alert state, the response to different spatial frequencies is bimodal. The highest optimal responses and firing rates are found to be at  $0,02\text{c/d}$  and  $0,3\text{c/d}$ . It has been showed that neurons in rat LGN have peak sensitivity for spatial frequencies between  $0,05$  and  $0,09\text{ c/d}$  (Lennie and Perry 1981). It might be a possibility that neurons in LGN and V1 respond strongly to non-overlapping frequencies, and in this way handle different aspects of the visual scene.

To induce anesthesia, the rats are given Midazolam, which is a muscle relaxant. It could be possible that the muscles controlling the lens or eyes are relaxed. Even though rats have no fovea, rats move their eyes to put objects into focus (Sefton et al. 2004). If the rat, under anesthesia, cannot adjust for details maybe the high spatial frequencies are not picked up as a visual stimuli, hence the low firing rate.



**Figure 26: Example of sinusoidal drifting gratings on the receptive field.** The big circle indicate the outer boundaries of a receptive field in V1, the inner circle indicate the receptive field center. A) A low spatial frequency will have few constituents covering the receptive field. In this particular case the neuron will simply be excited, if the neuron is an on-center cell. B) Stimuli with high spatial frequency will have more constituents covering the receptive field.

Another theory for why more neurons in alert rats respond to higher spatial frequencies than during anesthetized state could be related to the relative large receptive field found in V1 for rats (15-50°) (Shaw et al. 1975). Ganglion cells have receptive fields ranging from 6-26° (Heine and Passaglia 2011), and these cells converge onto their LGN thalamic targets, these targets also converge onto neurons in V1, meaning that V1 neurons must integrate the signals from many ganglion cells. When a stimulus with a high spatial frequency is presented, the receptive field of neurons in V1 will have information from many cycles at once.

Hypothetically, for a V1 neuron to code this stimulus, the signal from the cells earlier in the visual pathway must reach the neuron in a temporally precise manner. Greenberg and colleagues (2008) have shown a more synchronized firing of neurons in the visual cortex during anesthesia and preliminary results from our group show an increase in response latency for visual stimuli in anesthesia (Aasebø et al. 2014). If this is true also for cells early in the visual pathway, this could be the reason why neurons are not able to process the signal in an optimal manner in the anesthetized state. In comparison, a low spatial frequency will mainly represent an on/off signal, and thus less information needs to be converged and loss of information along the pathway is not detrimental for adequate response to the signal.

Previous findings in alert and anesthetized mice indicate a higher preferred spatial frequency of 0,08 c/d in V1 for alert mice (Andermann et al. 2011) than for anesthetized mice, which prefer a spatial frequency of 0,04 (Niell and Stryker 2008) (Figure 7). This comparison between responses in alert and anesthetized mice is not done considering the same population of neurons. Neither is the same method used to obtain information in alert state and anesthesia. However, it is interesting that these results might indicate the same tendencies in mice as I have found in rats.

A replacement towards preference for higher frequencies in alert state versus anesthetized state is also found in macaque LGN (M-cells) (Alitto et al. 2011). It is tempting to speculate that a similar shift in preference of the M-cells in thalamus of the rat could explain my findings as well.

### 4.2.3 Temporal Frequency

For temporal frequencies, most neurons analyzed in this project had an optimal response for 2 Hz in alert state, and 4 Hz in anesthesia (Figure 22A) when comparing all neurons. Girman and colleagues (1999) reported an optimal response of 3,88 Hz in anesthetized rats.

However, looking at the normalized ratio of mean firing rate of all neurons, it does not show a clear preference for 4 Hz in anesthesia. Even though most neurons had the highest response for 4 Hz in anesthesia, the relative change in firing rate, compared to the average firing rate, is not higher for 4 Hz. Due to a MatLab-error, 4 Hz in alert state and 8 Hz in anesthesia only had one session each. Thus an average between the two sessions measuring the same frequency could not be calculated. This might have influenced the result.

Using the similar selection criterion as for spatial frequency, seven neurons met the criterion; two in alert state, four in anesthesia and one neuron in both states. Most neurons had an optimal response for 2 Hz in both alert and anesthetized state (Figure 23B). The selective neurons in alert state had an increase in firing rate for 4 Hz, relative to the average firing rate. In anesthesia there is no clear increase in firing rate for any of the temporal frequencies (Figure 24A). This dataset, including only seven neurons, is too small to draw any conclusions.

A temporal frequency of approximately 8 Hz is found to be the cutoff for anesthetized rats (Girman et al. 1999), the cutoff could be higher for alert rats, and therefore higher frequencies should also be tested. If higher temporal frequencies had been tested, and more variables could be compared, the result could have been more consistent.

The LGN of macaque monkey seem to prefer higher temporal frequencies in alert state than in anesthesia (Alitto et al. 2011). The opposite result is found in this project. Neurons do also seem less selective for temporal frequencies than spatial frequencies. The reason for these findings could be that rats are dependent on detecting predators moving at high speed.

Detecting fast moving objects is a property which is essential for survival, and could be taken care of by primal structures in the brain, such as LGN or superior colliculus.

### **4.3 Future perspectives**

According to Niell & Stryker (2008), the preferred spatial frequency is higher for oriented simple cells than the median peak spatial frequency response for all neurons. It would be interesting to investigate if the preference for spatial frequencies is different between simple and complex cell in alert rats. It would also be interesting to sample more cells, so the preferred spatial and temporal preferences could have been investigated at the optimal orientation. More cells could also contribute to more statistically significant results. Putative inhibitory units respond optimally to low spatial frequencies in mice (Niell and Stryker 2008). This would have been interesting to investigate for alert rats. Girman and colleagues (1999) have reported a difference in preference across layers, by sampling more cells from different cell layers this could have been investigated also for alert rats. Since I find that alert rats respond stronger to higher spatial frequencies than in anesthesia, it is clear that there is a need to conduct future investigations in visual cortex with alert rats. Potentially, it could be specific neurons that have this increased response to higher spatial frequencies. This could have been uncovered in a larger dataset than my own. This could suggest a segregation of dorsal and ventral stream in the V1 that parallels the magnocellular and parvocellular pathways present up to the visual cortex.

### **4.4 Conclusions**

This project supports the notion that it is important to record from alert animals in order to figure out how information is processed in the brain. During anesthesia the spiking activity of the neurons are reduced by approximately 50% and the neurons do not respond strongly to stimuli with higher spatial frequencies as they do in the alert state. This suggests that when doing experiments in alert rats, the spatial frequency on the sinusoidal drifting gratings should be set to 0,3 c/d. This will give a greater response from the recorded single units.



## 5 APPENDIX

### 5.1 Staining for Nissl bodies using Cresyl Violet

Manually shake the specimen holder.

1. ddH<sub>2</sub>O for 1 minute
2. Cresyl Violet solution\* 3-10 minutes (depends on tissue and how fresh the solution is, the sections should obtain a dark blue color).
3. ddH<sub>2</sub>O for 1 minute
4. 70% ethanol for 2 minutes
5. 80% ethanol for 2 minutes
6. 90% ethanol for 2 minutes
7. 96% acetic acid for 30 seconds to 2 minutes, depending on color fading.
8. >100% ethanol for 2 minutes
9. Xylene for 2-10 minutes
10. Mount sections with cover slip and Entellan – leave in fume hood overnight.

Alcohol solutions are made with absolute ethanol, diluted in ddH<sub>2</sub>O.

\* 0,5g Cresyl Violet acetate

1,25 nL glacial acetic acid

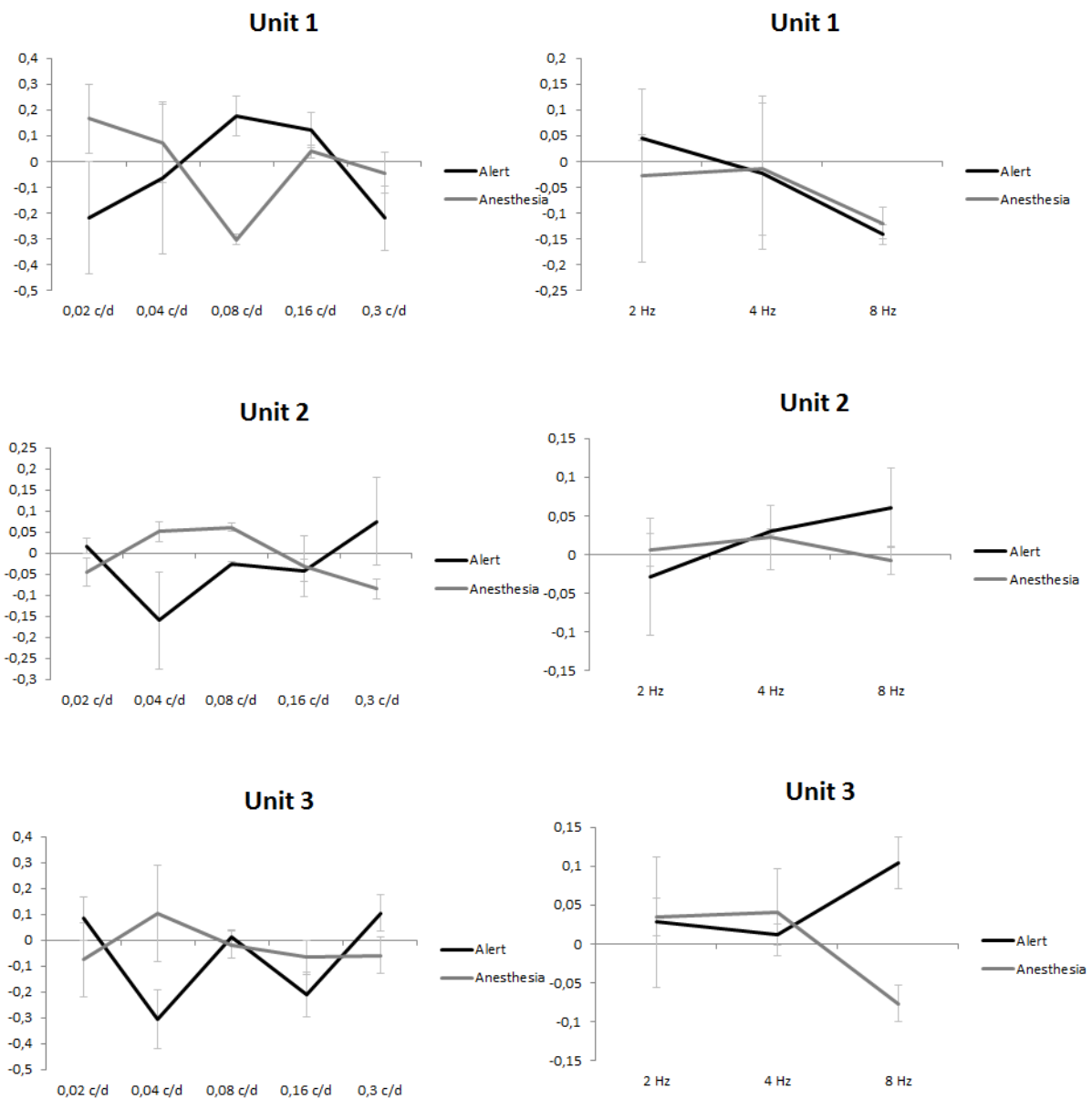
500 mL ddH<sub>2</sub>O

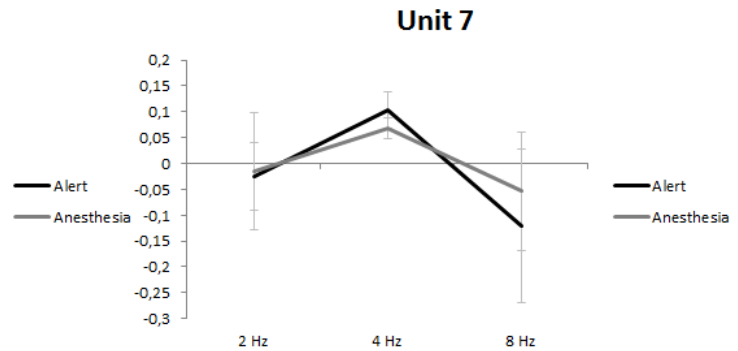
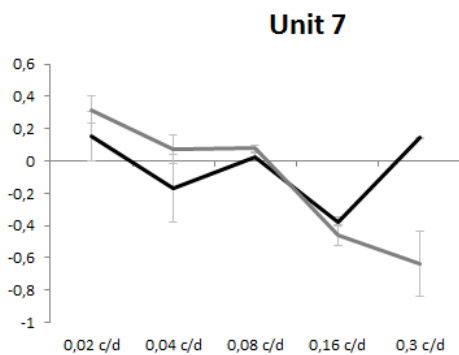
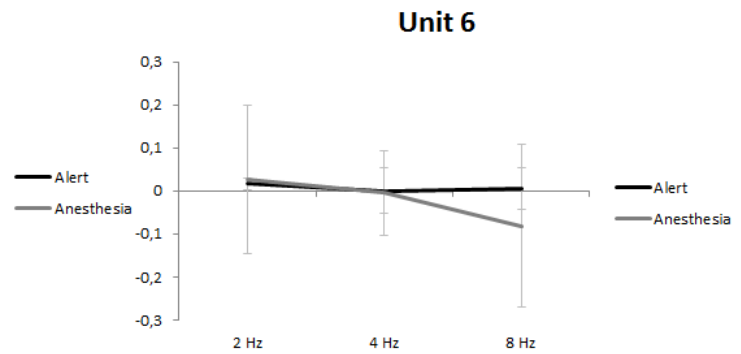
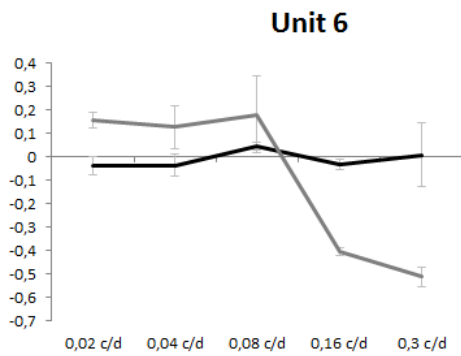
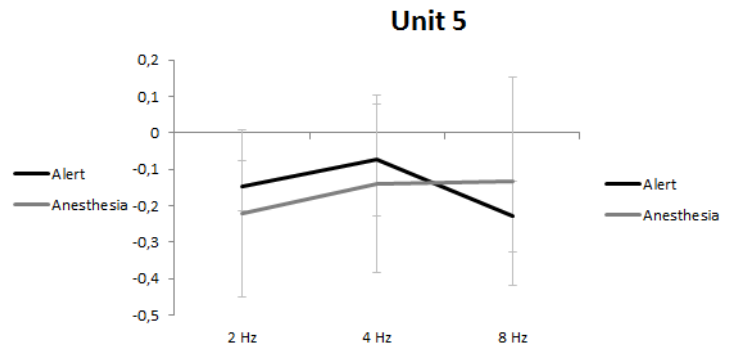
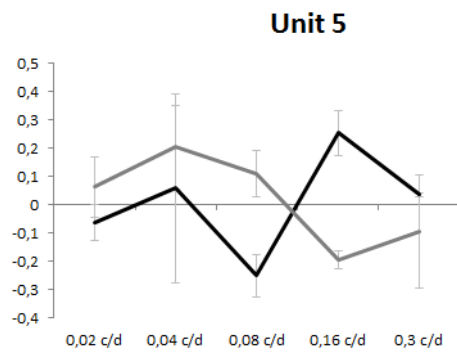
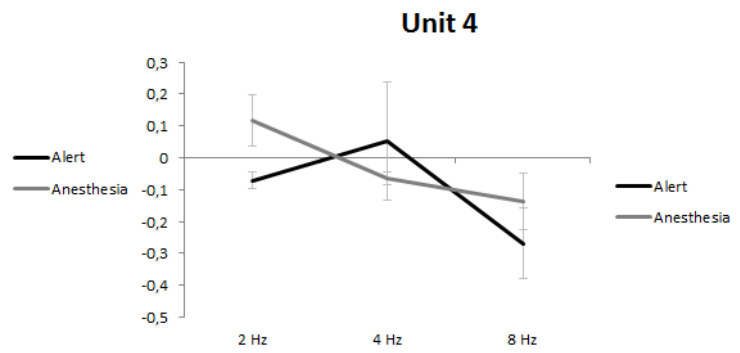
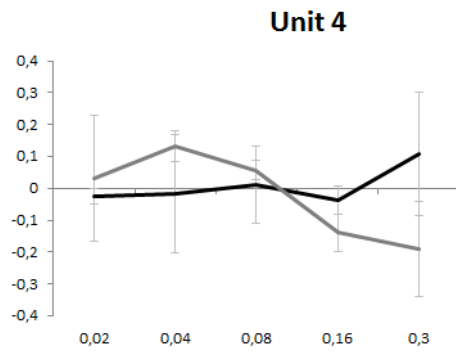
Heat to 60° and filter before use

## 5.2 Supplementary figures

### 5.2.1 Units recorded in experiment 1

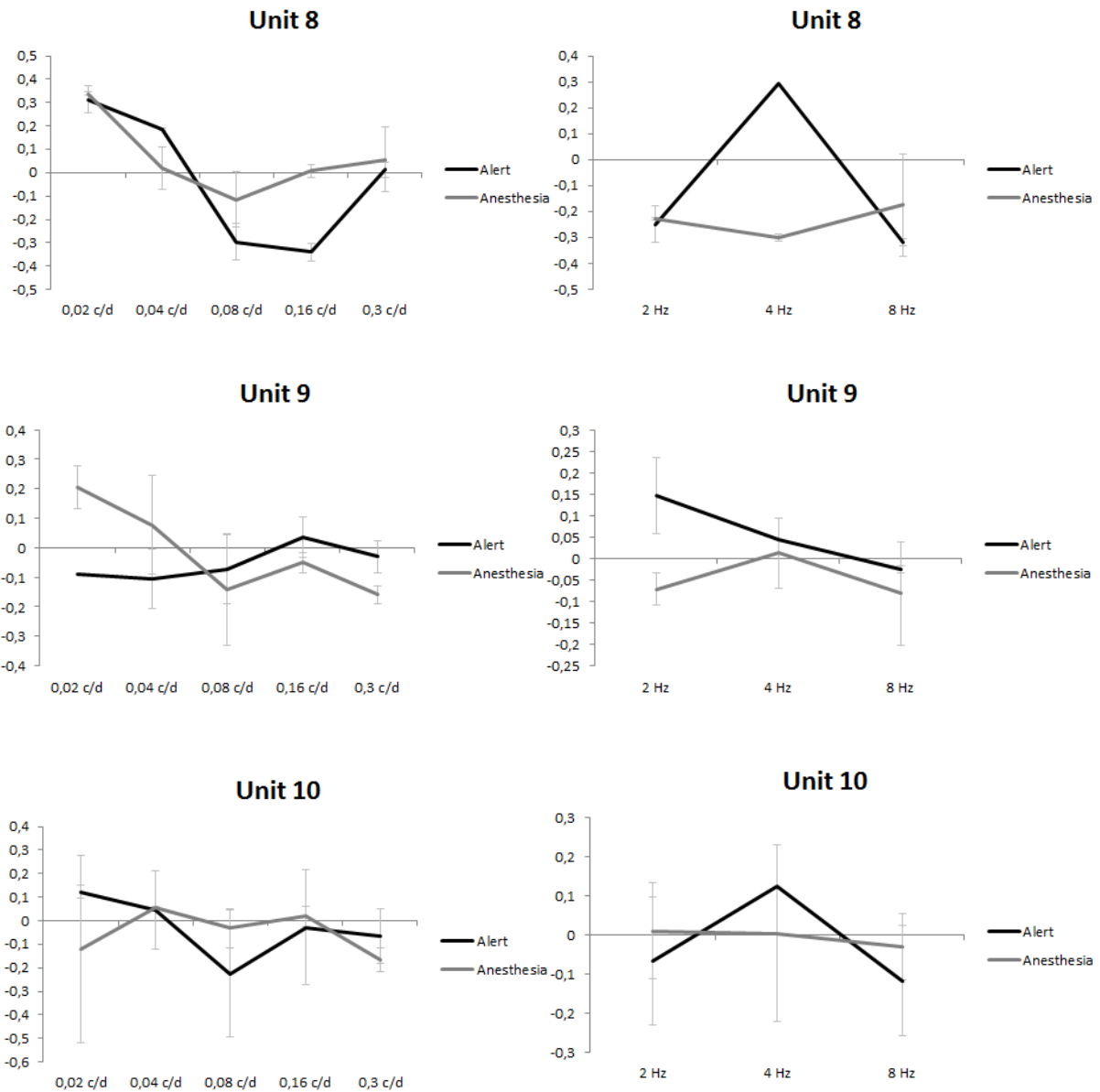
Normalized ratio for units recorded in experiment nr.1, rat 1444. Error bars indicate the difference between first and second recording. Figures to the left are responses to spatial frequencies, while figures to the right are responses to temporal frequencies.

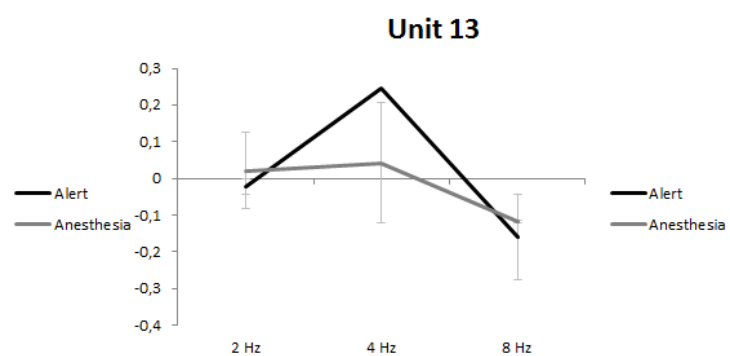
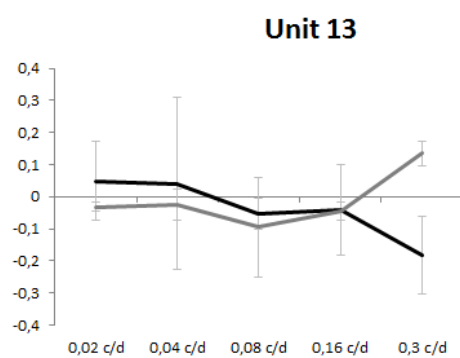
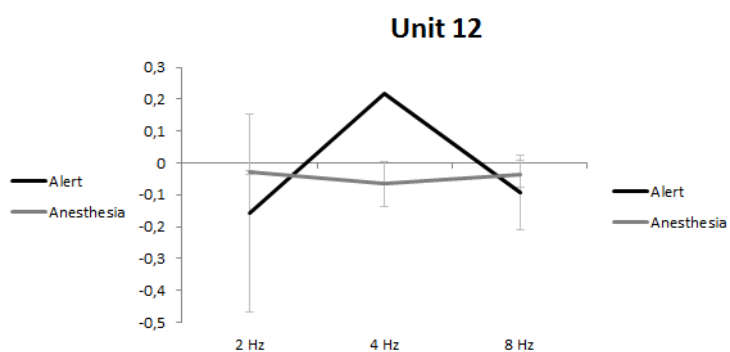
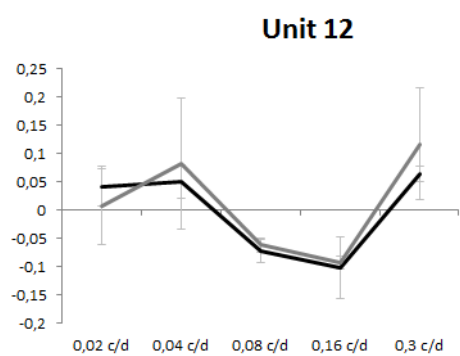
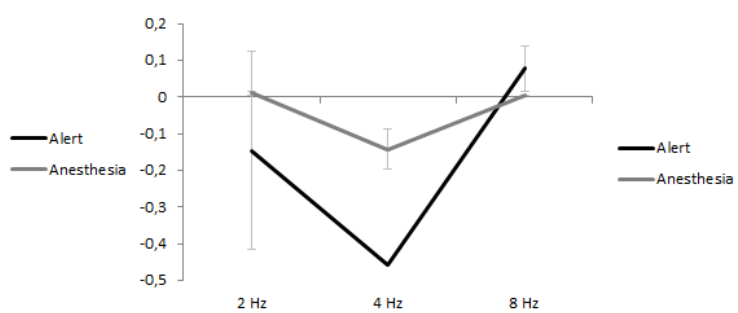
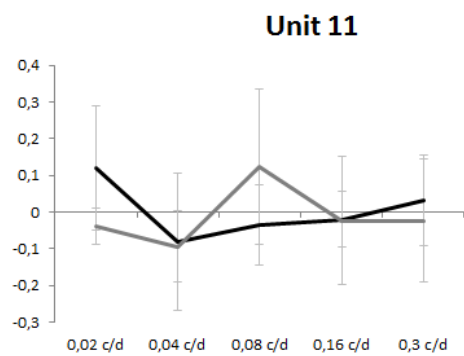




### 5.2.2 Units recorded in experiment 2

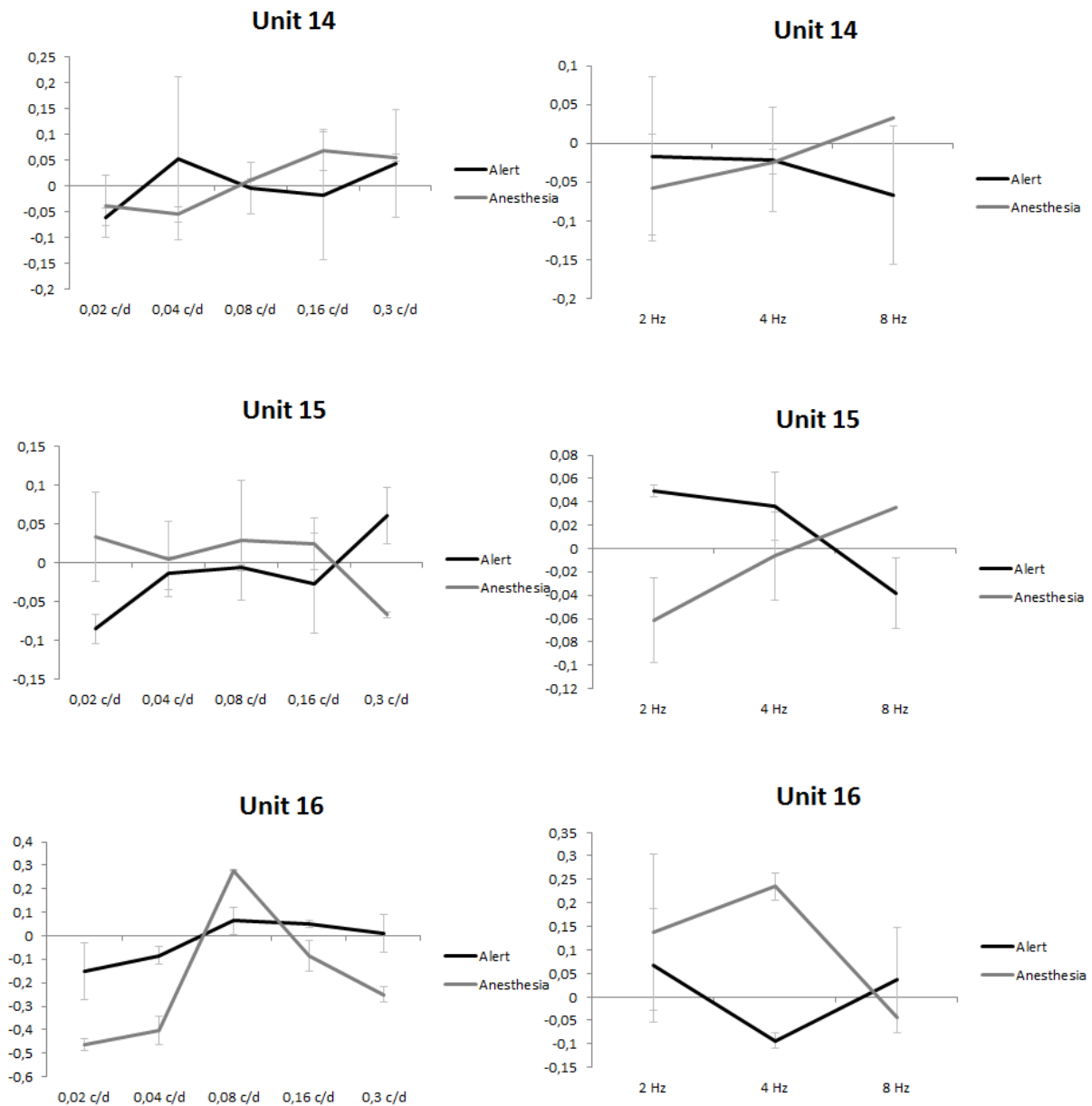
Units recorded in experiment nr.2, rat 1444. One session recording a temporal frequency of 4 Hz in alert state could not be analyzed in MatLab. This is the reason why these units do not have any error bars for 4 Hz.

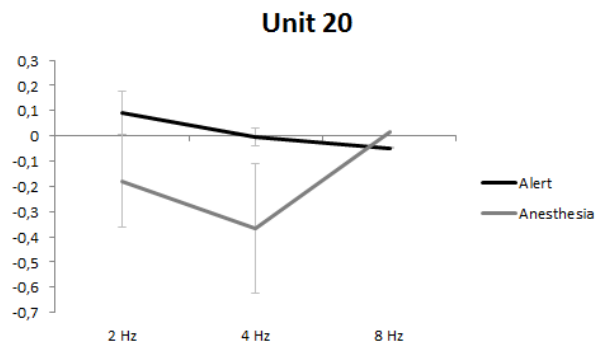
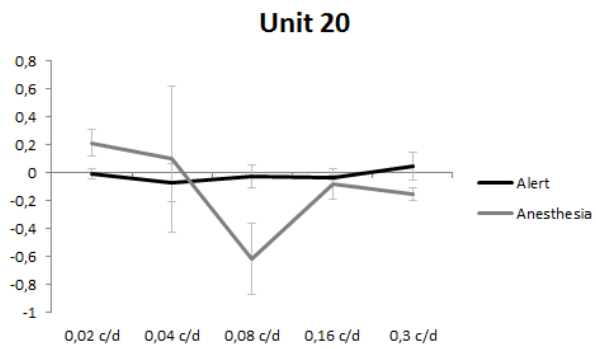
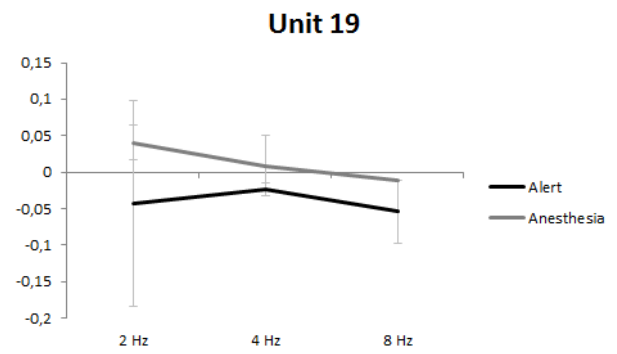
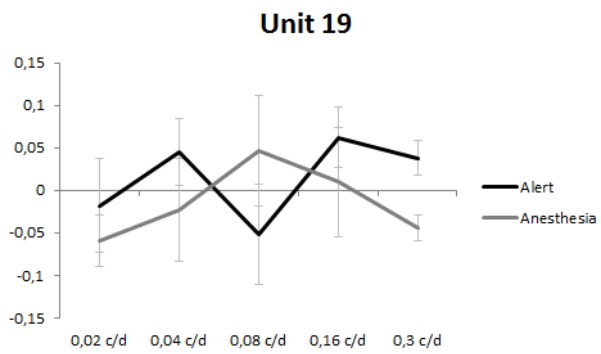
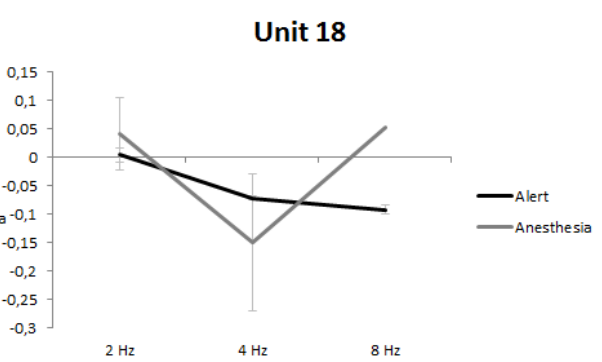
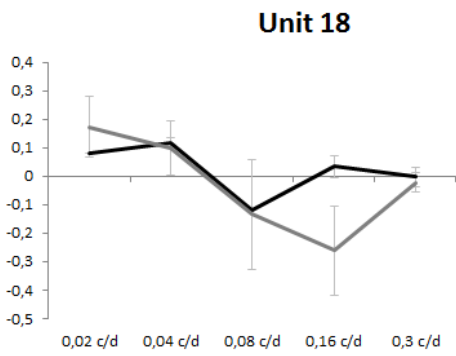
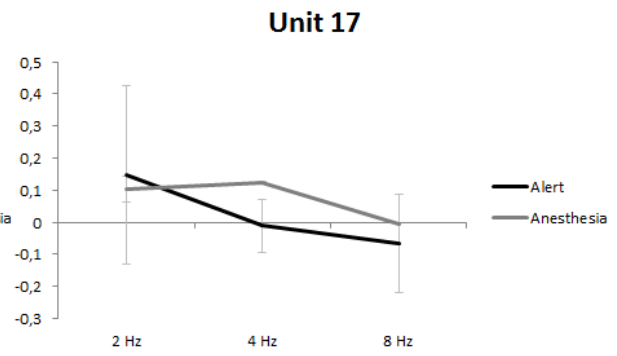
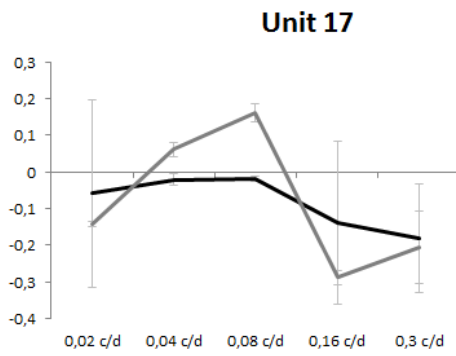


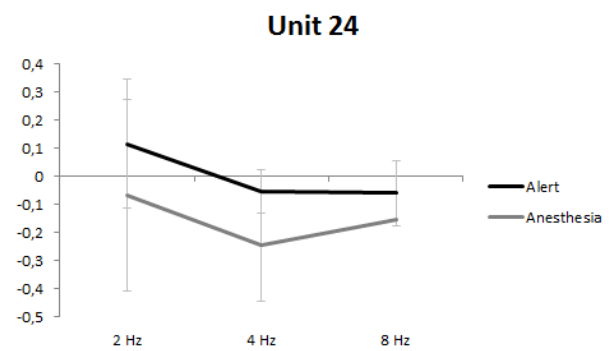
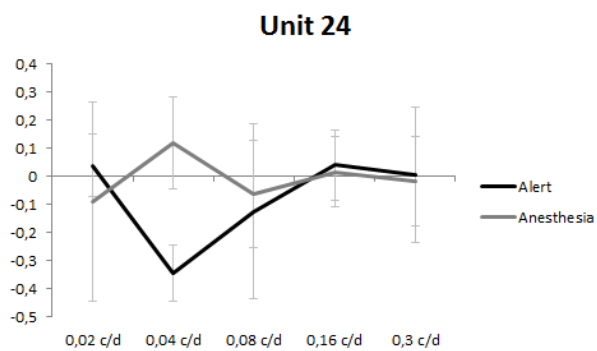
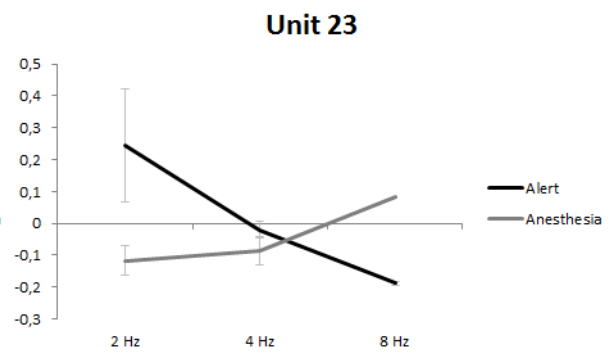
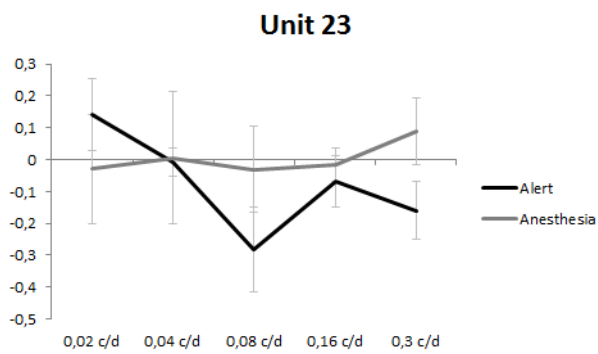
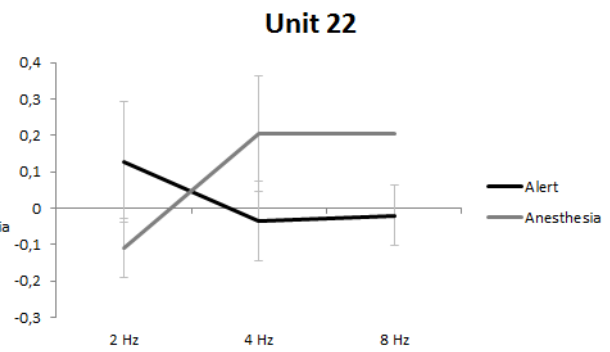
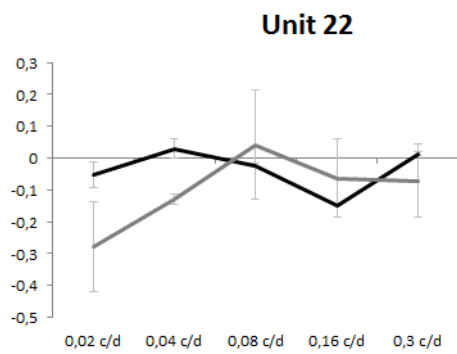
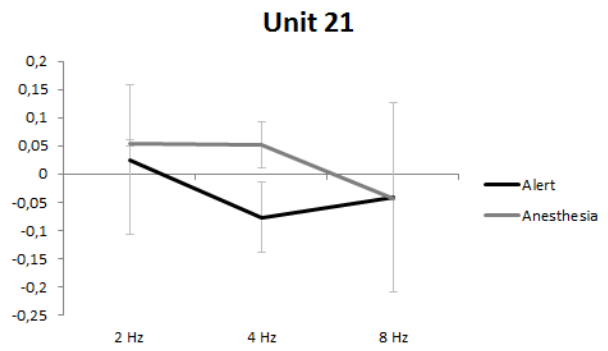
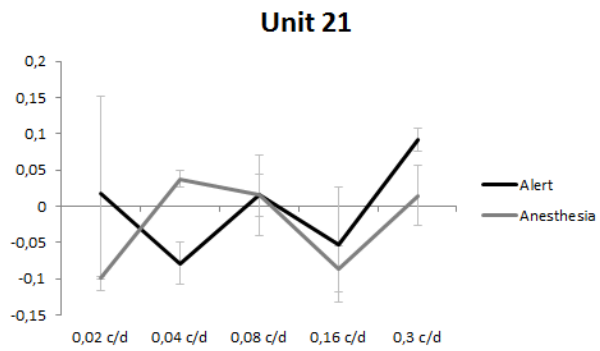


### 5.2.3 Units recorded in experiment 3

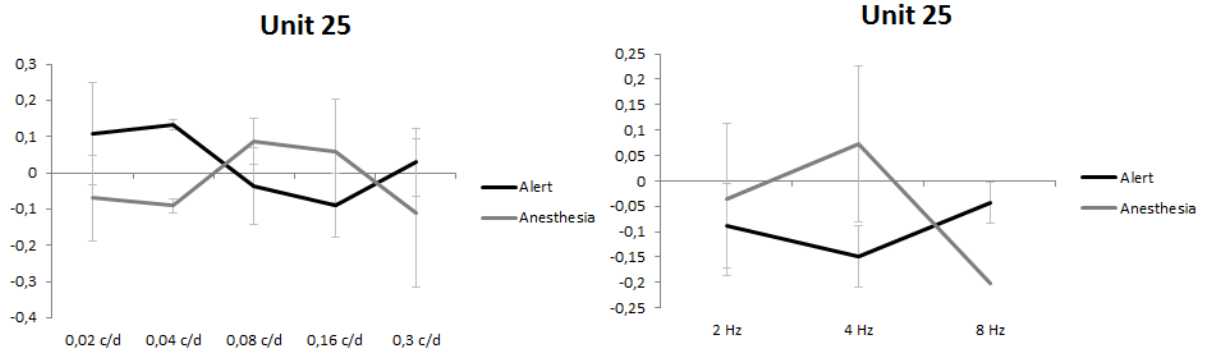
Units recorded in experiment nr. 3, rat 1446. One session recording a temporal frequency of 8 Hz in anesthesia could not be analyzed in MatLab. This is the reason why there is no error bars at 8 Hz in anesthesia.





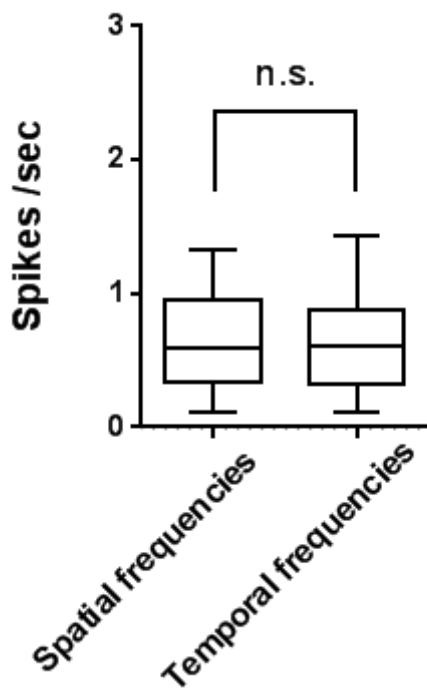






### 5.2.4 Supplementary analysis

Box and whiskers plot of the relative difference between the two sessions measuring the same frequency. There was no difference between the spatial frequency variations and temporal frequency variations. Box indicates median and upper and lower quartiles. Whiskers represent Tukey's Interquartile range.



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